Evolutionary history can shape belowground ecological interactions in eucalypts

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Declaration of originality

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Chapter 2 is submitted

John K. Senior, Julianne M. O'Reilly-Wapstra, Jennifer A. Schweitzer, Joseph K. Bailey, Brad M. Potts. Forest fire may disrupt plant-soil feedbacks. Journal of Plant Ecology.

The candidate was the primary author, undertook the fieldwork, planted and ran the glasshouse experiment as well as obtained, prepared and analysed the data. The candidate, JO'R-W and BMP contributed to developing the conceptual framework and design. Julianne O'Reilly-Wapstra, BMP, JAS and JKB assisted with refining the text.

Chapter 3 is prepared for submission

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Chapter 5 is published

John K. Senior, Brad M. Potts, Noel W. Davies, Rachel C. Wooliver, Jennifer A. Schweitzer, Joseph K. Bailey, Julianne M. O'Reilly-Wapstra. Phylogeny explains variation in the root chemistry of *Eucalyptus* species. Journal of Chemical Ecology, *in press*.

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Abstract

The influence of plant-microbial interactions on the structure and dynamics of native vegetation is gaining increasing attention. Plants may alter (i.e., 'condition') soil microbial communities with subsequent consequences for their performance via plant-soil feedbacks. Such feedbacks often vary in direction and magnitude among species and have been linked to the successional state, diversity and structure of temperate and tropical ecosystems. The drivers of variable conditioning and feedbacks are not clear, but recent studies suggest that plant evolutionary history may be a predictor of belowground ecological interactions. This thesis investigates whether plant evolutionary history can indeed explain plant-soil feedbacks using Tasmanian eucalypt species representing the subgenera, *Eucalyptus* and *Symphyomyrtus*, as well as the underlying genetic mechanisms.

In chapter 2, seedlings of a species from each subgenus, *E. globulus* and *E. obliqua*, were examined for responses to native soil inoculum that were consistent with plant-soil feedback, and whether feedbacks could be modified by wild fire. Soils were collected from beneath mature *E. globulus* or *E. obliqua* trees within native forest stands that had or had not been burnt by a recent wildfire and were subsequently used to inoculate seedlings of both species in a glasshouse experiment. *Eucalyptus globulus* displayed responses consistent with a positive plant-soil feedback, where seedlings performed better when inoculated with *E. globulus* as opposed to *E. obliqua* soils. However, this effect was only present when seedlings were inoculated with soils collected from unburnt as opposed to burnt stands, suggesting that fire removed the positive effect of *E. globulus* inoculum. These findings indicated that eucalypt species, and possibly subgenera, may differ in plant-soil feedbacks and these feedbacks can be influenced by external factors.

Chapter 3 tests whether feedbacks are a consequence of soil conditioning and whether there is a phylogenetic signal to these feedbacks. Seedlings of 14 Tasmanian eucalypt species from both subgenera were inoculated with soils conditioned by each of these species in a common garden. Conditioning and feedback effects were detected and shown to exhibit a significant phylogenetic signal. For each focal species, feedback was calculated as the slope of the linear regression

of its relative response to each conditioned soil against its phylogenetic distance from the soil conditioning species. Species from subgenus *Eucalyptus* performed better when inoculated with soils conditioned by more distant relatives (i.e., negative plant-soil feedback), while species from subgenus *Symphyomyrtus* either showed neutral or small positive responses. These results argued that plant evolutionary history can shape soil conditioning and plant-soil feedbacks.

In chapter 4, DNA was extracted from the same conditioned soils and sequenced to determine whether the eucalypt subgenera differentially conditioned soil microbes and whether conditioning was associated with phylogenetic signal in plant-soil feedbacks. Fungal community composition was found to differ between soils conditioned by each subgenus, indicating phylogenetic signal in the conditioning of fungal communities. Further, soils sampled from subgenus *Eucalyptus* species more frequently contained fungal taxa that exhibit pathogenic relationships with eucalypts. These taxa were associated with negative feedbacks to conditioned soils, presenting potential candidate organisms driving the negative responses of subgenus *Eucalyptus* to its own soils.

Chapter 5 examines species differences in root chemistry as a potential mechanism for conditioning of the soil microbial community, and ultimately, phylogenetic signal in plant-soil feedbacks. The concentrations of total phenolics, condensed tannins, carbohydrates, terpenes and formylated phloroglucinol compounds in the roots significantly varied among 24 Tasmanian eucalypt species studied from both subgenera. There was significant phylogenetic signal to this variation, with subgenus *Eucalyptus* roots containing higher concentrations of only total phenolics, while subgenus *Symphyomyrtus* roots contained higher concentrations of all other groups of compounds, especially, terpenes and formylated phloroglucinol compounds. Integration of these results with those from chapters 3 and 4, showed statistically significant relationships of root compounds with microbial taxa that were associated with feedbacks as well as the feedbacks themselves. These findings suggested that susceptibility of subgenus *Eucalyptus* species to soil pathogens and thus, negative feedbacks, may ultimately be related to root chemical traits.

This thesis contributes significantly to the field of plant-soil interactions. It provides further support for the use of evolutionary history as a predictor of plant ecological interactions. While plant-soil feedbacks have been associated with microbial

conditioning and recent work has suggested that soil conditioning can display a phylogenetic signal, this thesis provides the first evidence of a phylogenetic signal in both microbial conditioning and feedback responses. Thus, closely related species can condition similar microbial communities and respond to conditioned communities similarly, highlighting a putative mechanism driving phylogenetic structure to plant communities. This work encourages the continued investigation of phylogenetic structure in plant-soil interactions and holds the potential to increase our understanding of the mechanisms structuring plant communities and vegetation dynamics.

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Chapter 1
General introduction

Background

Ecologists have long sought to understand the factors explaining the structure and dynamics of native vegetation (Schulze and Mooney 2012; Tilman 1988; van der Maarel and Franklin 2012). Terrestrial plant communities can vary enormously in their structural complexity and diversity (Kier et al. 2005). At one end of this scale are communities such as, Arctic tundra, which are relatively species poor and structurally very simple (Tieszen 2012). At the other end are tropical ecosystems, with high levels of alpha and beta diversity and multilayered arrangements of herbs, shrubs, trees and epiphytes (Rafiqpoor et al. 2005). Over large spatial and temporal scales abiotic factors such as climate, age, environmental harshness, area, isolation, disturbance and environmental heterogeneity are strong predictors of plant communities (Rosenzweig 1995; Tilman and Pacala 1993). Locally, however, biotic factors may be more important including, competition and susceptibility to herbivory and pathogens (Johnson et al. 2012). One of the most influential hypotheses put forward to explain the maintenance of plant community structure and diversity was by Janzen (1970) and Connell (1971). They hypothesised that host-specific enemies accumulate around parental trees, diminishing the performance of conspecific seedlings, while allowing heterospecifics to grow relatively unhindered. This effect has been clearly demonstrated in several systems and may be driven by a range of organisms (Carson and Root 2000; Howe et al. 2002; Mangan et al. 2010). Among the biological factors influencing the structure and dynamics of native vegetation, plant-microbial interactions are presently gaining considerable attention (van der Putten et al. 2013).

Plant-soil feedbacks

Recent research shows that plant-soil interactions can drive the structure and dynamics of native vegetation. Plants affect soil properties or ‘condition’ soils through the addition of chemical compounds and organic matter, influencing water and nutrient availability as well as providing habitat and resources for

microorganisms (Ehrenfeld et al. 2005). Soil conditioning by plants may in turn have performance consequences for the individual, conspecifics or heterospecifics via plant-soil feedbacks (van der Putten et al. 2013). Positive plant-soil feedbacks occur when plants condition soils in a manner that promotes the performance of conspecifics, while negative plant-soil feedbacks occur when plants condition soils in a manner that is detrimental to the performance of conspecifics. Recent research shows that plant-soil feedbacks can be important drivers of plant succession, diversity and abundance in temperate and tropical ecosystems (Johnson et al. 2012; Kardol et al. 2006; McCarthy-Neumann and Kobe 2010a; b). For instance, Kardol et al. (2006) observed negative plant-soil feedbacks for early-successional species, neutral feedbacks for mid-successional species and positive feedbacks for late-successional species. Negative plant-soil feedbacks have been identified as a dominant force structuring patterns of diversity in tropical ecosystems (Liu et al. 2012; Mangan et al. 2010; Terborgh 2012). In these cases, the accumulation of host specific soil pathogens in close proximity to adult trees reduces the performance of conspecific seedlings, while allowing relatively unhindered performance of heterospecific seedlings (i.e., Janzen-Connell effect). Plant-soil feedbacks are also thought to contribute to the invasiveness of exotic species, where exotic species may exhibit negative plant-soil feedbacks in their natural range, but neutral or even positive plant-soil feedbacks in the range where they have become introduced (Callaway et al. 2004; Reinhart et al. 2003).

Plants may condition soils in a variety of ways that can subsequently feed back to influence performance (van der Putten et al. 2013). These include, changes to the physical components of soil (Ehrenfeld et al. 2005), nutrient immobilisation or depletion (Chapman et al. 2006) or by actively changing the composition of soil microbial communities (Klironomos 2002; Packer and Clay 2000). However, studies are increasingly pointing toward the conditioning of soil microorganisms as the predominant mechanism (Brinkman et al. 2010; Kulmatiski et al. 2008). Microbial conditioning has been implicated as the causal agent of plant-soil feedbacks through the use of inoculation and sterilisation procedures (Brinkman et al. 2010). For instance, seedlings may be inoculated with small quantities of conditioned soils or soil extracts to test for plant-microbe feedbacks, while excluding the influence of soil chemical properties (McCarthy-Neumann and Kobe 2010a). Alternatively, seedlings

may be grown in whole conditioned soils and sterilisation treatments applied to eliminate the soil biota in one half of the experiment and not the other (Liu et al. 2012). Plants may condition soil microbes through the exudation of root chemical compounds, growth phenology, water and nutrient use, as well as the quantity and quality of organic inputs to soils (Baetz and Martinoia 2014; Ehrenfeld et al. 2005). For instance, plant species with fast or slow growth rates tend to condition distinct microbial communities resulting from different plant traits (i.e., litter quality) that are associated with growth strategy (Baxendale et al. 2014; Orwin et al. 2010). In turn, soil communities may influence plants directly through interactions between plant roots and pathogens (Packer and Clay 2000) and mutualists (Klironomos 2002), as well as from indirect interactions with decomposing and mineralising soil organisms (Chapman et al. 2006; Hättenschwiler et al. 2005). However, studies have rarely investigated whether feedbacks are a community response or a response to individual organisms (but see Packer and Clay 2000).

Plants species often vary in the direction and magnitude of plant-microbial feedback effects (Kulmatiski et al. 2008; van der Putten et al. 2013). Many studies have shown plant-microbial feedbacks by using field collected soils (Brinkman et al. 2010). While conditioning effects detected in field collected soils may reflect soil conditioning by plant species, these effects may be confounded by shared soil niches of plant species and soil organisms or variable plant age (i.e., timeframe of soil conditioning). To remove such confounding effects, studies have grown species in a uniform soil media or in replicated and randomised field experiments to conditioned soils (Bezemer et al. 2006; Kardol et al. 2007; Perkins and Nowak 2012; Smith and Reynolds 2012). These studies show that species can differentially condition soil microbial communities and conditioned communities may subsequently influence the performance of the conditioning species or heterospecifics. For instance, Kardol et al. (2007) found that six grass and forb species generally experienced negative plant-soil feedback to conspecific conditioned soils. In contrast, Smith and Reynolds (2012) found that a native ground cover species, *Asarum canadense*, displayed a neutral feedback, while an invasive species, *Euonymus fortune*, displayed a positive feedback, possibly contributing to its invasiveness. Such studies demonstrate that plant species can display variable plant-soil feedbacks, with likely consequences for the behaviour of species within a community context. Thus, approaches that provide

generality to plant-soil feedbacks may be essential to understanding plant community structure and succession.

Plant evolutionary history may shape plant-soil feedbacks

Evolutionary history is emerging as an important predictor of plant ecological interactions. Plant traits commonly display phylogenetic signal, where close relatives tend to resemble each other more than they resemble species drawn at random from the phylogenetic tree (Blomberg and Garland 2002). Traits implicated in plant ecological interactions may also show such phylogenetic signals (Agrawal et al. 2009a; Pearse and Hipp 2009). Thus, it is not unexpected that related species which share traits, as a result of shared evolutionary history, may also share similar interactions with their abiotic and biotic environment. Indeed, the evolutionary relationships among plant species can predict a range of ecological interactions including, susceptibility to herbivores and pathogens (Gilbert and Webb 2007; Hill and Kotanen 2011; Pearse and Hipp 2009). Such phylogenetic signals may be used to predict the susceptibility of plant species to exotic pests and pathogens (Gilbert et al. 2012) as well as their invasive potential in novel ranges (Strauss et al. 2006). Recent studies provide evidence that plant evolutionary history may also predict belowground ecological interactions, including susceptibility to herbivores and fungal pathogens (Liu et al. 2012; Vannette and Rasmann 2012) as well as plant-mycorrhizal associations (Reinhart et al. 2012). Thus, evolutionary history may potentially predict plant-soil feedbacks.

There is some evidence to suggest that forest communities may be phylogenetically structured (Liu et al. 2012), where neighbouring tree species are less phylogenetically related than expected by chance. Experimental evidence argues that these patterns are driven by a 'phylogenetic Janzen-Connell effect' (Liu et al. 2012), where the performance of seedlings in conditioned soils is dependent upon the degree of phylogenetic relatedness between focal and conditioning species. For example, Liu et al. (2012) found that the relative survival of eight sub-tropical tree species in native soils collected from beneath co-occurring *Castanopsis fissa*, increased with increasing phylogenetic distance of the focal species to *C. fissa*. The application of fungicide to *C. fissa* soils removed this effect, implicating fungal pathogens as the causal agent of feedbacks. Only recently have studies considered testing for phylogenetic signal in plant-soil feedbacks by varying both the test and

soil conditioning species (Anacker et al. 2014). By doing so, phylogenetic signal in soil conditioning may also be tested. If plant lineages differentially condition soil communities, the susceptibility of species to a phylogenetic Janzen-Connell effect may itself exhibit phylogenetic signal.

Eucalyptus

Eucalyptus L' Hérit (Myrtaceae) is a large genus of over 700 species (Brooker 2000) and is widely distributed, occurring in Australia, Papua New Guinea, Timor, Sulawesi, and the Philippines. In Australia, the genus dominates many ecosystems including, subalpine woodlands, cool and warm temperate forests, rainforests and tropical savannahs (Williams and Woinarski 1997). Given the frequency of fire in Australian landscapes, many species are fire adapted or are even dependent on forest fire for establishment (Gill 1997). Thus, having evolved under a large range of climatic and edaphic conditions, eucalypts vary enormously in growth form, from giant forest to dwarf coastal trees and stunted, multi-stemmed, "mallee" forms in semi-arid areas (Brooker 2000). While the genus is easily distinguished by its characteristic leaf, floral and fruit morphologies, there is a large amount of quantitative variation and homoplasy (convergence/parallelism) in phenotypic characters, both among and within species (Pryor and Johnson 1981; Pryor and Johnson 1971). To further complicate matters, eucalypts readily hybridise, resulting from incomplete reproductive isolation among species (Griffin et al. 1988; Pryor and Johnson 1981; Pryor and Johnson 1971). Historically, these factors have made the reconstruction of the phylogenetic history of eucalypt species difficult, particularly at levels below subgenus (Steane et al. 2011 and references therein). However, recent studies have made significant advances in elucidating phylogenetic relationships below this level (McKinnon et al. 2008; Steane et al. 2011).

As the dominant genus of Australia, *Eucalyptus* is of great ecological importance. Eucalypts support a range of dependent organisms including, foliar pathogens and aboveground invertebrate and mammal herbivores (Matsuki et al. 2011; O'Reilly-Wapstra et al. 2010; Wingfield et al. 2008). Thus, eucalypt foliage is chemically defended by a range of plant secondary metabolites, most notably, terpenes, cyanogenic glycosides and phenolics (Gleadow et al. 2003; Mann et al. 2012; McKiernan et al. 2014), including formylated phloroglucinol compounds (Moore et al. 2005). These chemical defences often display substantial genetic variation,

occurring between subgenera, species and populations (Dungey et al. 2000; Eschler et al. 2000; Humphreys et al. 2008; Wallis et al. 2002) and the consequences of this variation for dependent organisms have been well studied (e.g., Lawler et al. 1998; Matsuki et al. 2011; O'Reilly-Wapstra et al. 2010). However, the genetic basis of eucalypt belowground ecological interactions has received little attention in comparison. There is some evidence to suggest that eucalypt species may differentially condition soil chemical and biological properties (Anderson et al. 2013; Sayad et al. 2012), but whether soil conditioning by eucalypts can feed back to influence performance is not known. Eucalypts also often form symbiotic relationships with ectomycorrizal fungi and are susceptible to a range of belowground organisms (Noble 1989; Wingfield et al. 2008), including the fungal pathogen *Phytophthora cinnamomi*. The relationships of eucalypts with these organisms can differ among species or subgenera (Noble 1989). For example, eucalypt species belonging to subgenus *Symphyomyrtus* are generally resistant to *P. cinnamomi*, whereas subgenus *Eucalyptus* species have been found to be more susceptible (Podger and Batini 1971; Tippett et al. 1985). These studies suggest that eucalypt species may display plant-soil feedbacks, with possible outcomes for the structure and dynamics of Australian vegetation.

The southern island state of Tasmania is home to 30 native species of eucalypt, belonging to the two larger eucalypt subgenera, *Eucalyptus* (13 species) and *Symphyomyrtus* (17 species). These species occur in a diverse range of habitats, from sea-level to the high-altitude tree-line, and vary in growth form, from a small alpine shrub (*Eucalyptus vernicosa*) to giant forest trees, including the world's tallest angiosperm (*Eucalyptus regnans*) (Williams and Potts 1996). Sixty percent of the species are endemic to the island of Tasmania, two or which are threatened and in decline (Jones et al. 2005; Sanger et al. 2011). The island also contains several species that are of great economic importance to the forestry industry, including *E. obliqua*, *E. delegatensis* and *E. regnans* (Baker and Read 2011). As on mainland Australia, Tasmania's eucalypt species dominate many ecosystems and are vital to a range of dependent organisms. Members of each subgenus species often co-occur in mixed stands that tend to include at least one species from each subgenus (Austin et al. 1983; Davidson and Reid 1980; Duff et al. 1983). Many key ecological differences between these subgenera are suggested to maintain this coexistence,

including differences in germination, growth, biomass allocation and susceptibility to mammalian herbivores, insect pests and fungal pathogens (Eschler et al. 2000; Noble 1989; Stone et al. 1998; Wallis et al. 2010). There is also evidence that the subgenera differ in their relationships with soil pathogens and mycorrhizae (Noble 1989; Podger and Batini 1971), suggesting that phylogenetic differences in plant-soil feedbacks should also be considered as a potential driver of stand structure and dynamics.

Overview of thesis

Given the ecological importance of the genus *Eucalyptus*, there is surprisingly little information regarding the ability of eucalypt species to differentially condition soil biological and chemical properties (but see Anderson et al. 2013; Sayad et al. 2012) and no studies could be found on how these conditioning effects feed back to influence eucalypt performance. However, an extensive literature on other plant systems shows that plant-soil feedbacks are widespread and vary among species, with important consequences for plant community structure and dynamics (van der Putten et al. 2013). This highlights the need for approaches that can provide generality to observations of soil conditioning and plant-soil feedbacks. So far, there is some evidence showing the importance of phylogenetic distance between focal and conditioning species in determining feedbacks (Liu et al. 2012), but little information regarding the use of plant evolutionary history in predicting soil conditioning or plant-soil feedbacks.

In this thesis, I investigate plant-soil feedbacks in 14 Tasmanian eucalypt species representing the subgenera, *Eucalyptus* and *Symphyomyrtus*, and the influence of forest fire on feedbacks and whether evolutionary history can predict such feedback effects as well as the underlying genetic mechanisms. There are three experiments: (i) a plant-soil feedback experiment using field collected soils from beneath *E. globulus* and *E. globulus* trees in burnt and unburnt stands, (ii) a common garden experiment consisting of 14 of the Tasmanian eucalypt species and (iii) a plant-soil feedback experiment including the same 14 eucalypt species. Using these experiments, I aimed to determine whether:

1) the responses of eucalypt species to native soil inoculation are consistent with plant-soil feedbacks and whether this is modified by the effect of fire (Chapter 2);

2) plant-soil feedbacks can be driven by soil conditioning and exhibit a phylogenetic signal at the species level (Chapter 3);

3) soil conditioning is associated with changes in microbial communities and whether such changes can explain phylogenetic signals in plant-soil feedbacks (Chapter 4); and whether

4) there is a phylogenetic signal in root chemistry, which could provide a mechanism for conditioning of the soil microbial community and ultimately drive the phylogenetic signals in plant-soil feedbacks (Chapters 5 and 6).

These four aims represent four experimental chapters and these are presented as stand-alone studies, as scientific journal articles. Each chapter contains of an introduction, summarising the relevant literature and identifying areas in need of research, a results section and a discussion of the findings and conclusions of the study. In chapter 6, a synthesis of the major findings of the experimental chapters, their implications in regard to the relevant literature and directions of future research are presented.

Chapter 2
Forest fire may disrupt plant-soil feedbacks

This chapter is submitted to the Journal of Plant Ecology.

John K. Senior, Julianne M. O'Reilly-Wapstra, Jennifer A. Schweitzer, Joseph K. Bailey, Brad M. Potts. Forest fire may disrupt plant-soil feedbacks.

Summary

Plant-soil feedbacks are major drivers of plant community structure and dynamics. Often these feedbacks are driven by variable soil biota resulting from a history of plant conditioning. However, other factors besides plant conditioning can influence soil communities and thus potentially interact with plant-soil feedbacks. We tested for plant-soil feedbacks in two *Eucalyptus* species, *E. globulus* and *E. obliqua*, and the influence of forest fire on these feedbacks.

We collected soils from a native eucalypt forest on the Forestier Peninsula, Tasmania, Australia. Soils were collected from beneath mature *E. globulus* or *E. obliqua* trees within stands that had or had not been burnt by a recent wildfire and were subsequently used to inoculate seedlings of both species in a glasshouse experiment. We hypothesised that (i) *E. globulus* and *E. obliqua* would display plant-soil feedbacks and (ii) feedbacks would differ between burnt and unburnt stands. For each species, mixed linear models tested for differences in seedling performance in response to inoculation with conspecific (home) versus heterospecific (away) soils that had been collected from either unburnt or burnt stands.

Eucalyptus globulus displayed responses consistent with a positive plant-soil feedback, where seedlings performed better when inoculated with home versus away soils. However, this effect was only present when seedlings were inoculated with unburnt soils, suggesting that fire removed the positive effect of *E. globulus* inoculum. These findings show that species differ in plant-soil feedbacks and these feedbacks can be influenced by external factors with possible implications for plant community structure and dynamics.

Introduction

Soils contain a vast diversity of organisms including microbial pathogens, saprobes and symbionts of plant roots as well as root herbivores, all of which may have profound impacts on plant performance (Kulmatiski et al. 2014; Van der Heijden et al. 2008a). Plant species may differentially 'condition' soil communities through the addition of chemical compounds and organic matter, thus modifying habitat and resources (Ehrenfeld et al., 2005). The conditioned communities may in turn have performance consequences for the individual, conspecifics or heterospecifics via plant-soil feedbacks (Kulmatiski et al. 2008; van der Putten et al. 2013). Plant-soil feedbacks can have important landscape-level consequences for plant coexistence, diversity and succession in temperate and tropical ecosystems (Johnson et al. 2012; McCarthy-Neumann and Kobe 2010a; b). In diverse tropical ecosystems, negative plant-soils feedbacks are thought to maintain high levels of tree diversity (Mangan et al. 2010; McCarthy-Neumann and Kobe 2010a; Terborgh 2012). In these cases, the performance of conspecific seedlings is reduced in close proximity to adult trees due to an accumulation of host-specific soil pathogens, while the performance of heterospecific seedlings is relatively unhindered. Plant-soil feedbacks can also play an important role in plant community succession. For example, Kardol et al. (2006) observed negative plant-soil feedback for early-successional species, neutral feedbacks for mid-successional species and positive feedbacks for late-successional species. However, factors besides conditioning by plants may also influence soil communities and thus potentially interact with plant-soil feedbacks.

Fire is an important disturbance event in many ecosystems with important consequences for vegetation structure and dynamics (Bond and Van Wilgen 2012). Despite the obvious effects of forest fire (e.g., removal of aboveground vegetation and nutrient release), fire may also influence soil communities (Dooley and Treseder 2012; Xiang et al. 2015; Xiang et al. 2014). This may occur directly through heat induced mortality or indirecty via changes to soil physical and chemical properties as well as the removal of aboveground vegetation (Dooley and Treseder 2012). As with conditioning by plants, these fire-induced changes to soil communities may in turn have consequences for plant performance (Allen et al. 2003; Allen et al. 2005; Soteras et al. 2013). For example, Allen et al. (2005) found that the growth of six tree species in a field study differed in their response to inoculation with soils

collected from a mature forest and an adjacent stand that had been recently burnt. This suggests that forest fire may potentially influence plant-conditioned soils, possibly resulting in the disruption of plant-soil feedbacks. While there is some evidence to suggest that soil type and nutrient availability may influence the sign and magnitude of feedback effects (Manning et al. 2008; Schradin and Cipollini 2012), the influence of fire is relatively unknown. Knowledge of the interactive effects of plant-soil feedback and fire may help disentangle the drivers of plant community structure and dynamics in ecosystems that experience both wildfire and plant-soil feedback.

The genus *Eucalyptus* is planted worldwide in forest plantations and is the dominant native genus of many Australian ecosystems (Williams and Woinarski 1997). The genus is of economical and ecological importance and, therefore, important to determine what factors drive the performance of these species. There are some reports of eucalypt species differentially influencing soil chemical properties (Orozco-Aceves et al. 2015; Sayad et al. 2012), but whether eucalypt species differentially influence soil biotic communities and display feedbacks has received little attention (but see Orozco-Aceves et al. 2015). *Eucalyptus* species are generally dependent on forest fire for establishment (Gill 1997). While fire is known to affect soil physical and chemical properties in eucalypt forests (see Certini 2005), it is also possible that fire may indirectly influence eucalypt growth via altering soil microbial community composition. Herein, we investigated whether two *Eucalyptus* species, *E. globulus* (subgenus *Symphyomyrtus*) and *E. obliqua* (subgenus *Eucalyptus*) display plant-soil feedbacks and whether wildfire influences feedback effects. We utilised an inoculum-based method to test for plant-microbe feedbacks and the influence of wildfire, while excluding the influence of soil chemical properties (Mangan et al. 2010; Maron et al. 2014; van Grunsven et al. 2007). This method is common in plant-soil feedback studies, where soil treatments are often observed in the field and small amounts of collected soil samples are used to inoculate seedlings and test for conditioning effects (Kulmatiski et al. 2008). We collected soils from a native eucalypt forest on the Forestier Peninsula, Tasmania, Australia. Samples included soils collected from beneath mature *E. globulus* or *E. obliqua* trees within stands that had or had not been burnt by a recent wildfire. These samples were subsequently used to inoculate seedlings of both species in a fully factorial glasshouse experiment.

We tested the hypotheses: (i) *E. globulus* and *E. obliqua* would display plant-soil feedbacks and (ii) feedbacks would differ between burnt and unburnt stands.

Materials and methods

Soil collection

We sourced soil inoculum from a native eucalypt forest on the Forestier Peninsula in South-East Tasmania, Australia (42°56'12.06"S, 147°53'40.92"E). The collection site was located in mature, damp eucalypt forest (up to 50 m tall) with an understory dominated by *Pomaderris apetala*, *Bedfordia salicina* and *Olearia argophylla*. Soils were brown/red ferrosol derived from dolerite with moderately well-drained clay loams lying over medium to heavy clays; the area receives an average rainfall of approximately 900 mm per annum (Neyland et al. 1999). In January 2013 a wildfire burnt through the study area leaving a mosaic of burnt and unburnt patches. Within burnt patches, the understory and herbaceous layers were mostly removed and the lower trunks of mature eucalypts were burnt, but the fire did not reach the canopy.

One year following fire, we collected soils from six forest patches (detailed below) to use as inoculum and test for the influence of tree species and fire on soil biota. To avoid any major changes in soil characteristics, forest patches were located no more than 250 m away from one another. Soils were sampled from beneath mature *E. globulus* and *E. obliqua* individuals (tree species) across two stands each that had or had not experienced recent burning (burning), giving four soil treatments (2 tree species x unburnt/burnt = 4 treatments). Soils were sampled beneath each species in a pure stand (dominated by a single species) and a mixed stand (codominated by both eucalypt species) to minimise the influence of any differences in eucalypt microhabitat on soil biota within the site. For each treatment combination, soils were sampled from beneath 20 mature trees, giving a total of 80 separate samples. Three soil cores to 15 cm of depth were taken 1-2 m away from each individual tree and bulked. Soils were placed in a cooler immediately after sampling and the soil corer was washed with detergent and rinsed with water between each sample to limit cross contamination of soils. Samples were then stored at 4 °C for no more than 48 h before being used to inoculate seedlings. These samples were kept separate throughout the experiment and referred to as inocula.

Preparation of plant material

Open-pollinated seed was collected from ten mature individuals of *E. globulus* and *E. obliqua* located within 10 km of the site. The seed lot collected from each individual tree was kept separate throughout the experiment and is hereafter referred to as a 'family'. Seed capsules of each family were dried at 40 °C for 72 h and sieved to collect seed. The seed of each family was germinated in sterile vermiculite in sterile plastic trays for three weeks until individuals of each species had developed their first pair of true leaves.

Plant-soil feedback experiment

To test for eucalypt feedback to soil biota and the influence of fire on plant-soil feedbacks, seedlings of each species were grown in potting soil inoculated with each field soil. The potting soil used consisted of eight parts composted pine bark and three parts coarse river sand with added macro- and micronutrients from Osmocote® For Natives low phosphorus, slow-release fertiliser (Scotts® Australia Pty Ltd, Baulkham Hills, NSW, Australia). The potting mix included approximately 1.92, 0.16, 1.09 g Kg^{-1} dry mass of nitrogen (N), phosphorus (P) and potassium (K), respectively, that is slow-released over approximately six months. No extra fertiliser was applied during any part of the experiment. For each of the 80 inocula, four forestry tubes were three-quarters filled with potting soil. A small amount of inoculum (approximately 5% potting soil volume) was placed on the surface of all four forestry tubes to ensure that seedlings had first contact with inoculum. Two seedlings of each eucalypt species were then transplanted directly into the inoculum of two separate forestry tubes each. With 80 inocula by two eucalypt species by two seedlings per species, the design consisted of a total of 320 forestry tubes. Forestry tubes were organised into a randomised complete block design, where each combination of soil treatment and seedling species was represented once in each of 20 blocks.

After approximately three months of growth, the survival of seedlings was recorded and surviving seedlings were destructively harvested to test for the effects of inocula. Seedlings were carefully removed from their forestry tubes, with soil gently shaken and massaged off the roots. The roots were rinsed to wash off any remaining soil. Seedlings were cut at the root collar to yield above- and belowground biomass. The

above- and belowground plant parts were placed in separate paper bags, dried at 60 ℃ for 48 h and then weighed. The belowground biomass was divided by aboveground biomass to yield root to shoot ratio and both above- and belowground biomass were summed to yield total biomass.

Statistical analysis

All statistical analyses were conducted using the statistical package SAS (version 9.2, SAS Institute Inc., Cary USA). To test for plant-soil feedbacks and the influence of fire, mixed linear models were fitted analysing for differential survival and growth responses of *E. globulus* or *E. obliqua* seedlings to inoculation treatments using PROC GLIMMIX. Biomass traits were analysed assuming a Gaussian distribution of residuals whereas survival was analysed using a binomial model with a logit link function. Models were fitted separately for each seedling species. Models included the fixed terms tree species (*E. globulus*/*E. obliqua*), burning (unburnt/burnt forest patch) and their interaction. The random term inoculum within the interaction of soil species and fire was used to test for inoculum treatment effects. Block was included as a random factor in all models to account for spatial variation within the glasshouse design and seed lot was also included as a random term to account for variation among the seed lots of each species. Plant-soil feedback was indicated by a significant effect of tree species (home versus away soils) on the survival or biomass of each seedling species, while a significant interaction between tree species and burning indicated an influence of burning on plant-soil feedback.

Residuals were tested for assumptions of normality and homoscedasticity for the biomass traits and appropriate transformations were applied to meet the Shapiro-Wilk test and diagnostic graphical representations were also checked. All biomass traits were log transformed.

To present feedback effects, the log-transformed ratio of response (Hedges et al. 1999) to inoculation with home versus away soils collected from either burnt or unburnt soils was calculated for the total biomass of each species. Specifically, for each species, we took the logarithm of the averaged total biomass of seedlings when inoculated with home soils divided by the average total biomass of seedlings inoculated with away soils (Brinkman et al. 2010). Response ratios were calculated

from the least squares means of inoculum treatment groups obtained from mixed linear models (above).

Results

In support of the first hypothesis, eucalypt species displayed variable responses to inoculation treatments, some of which were consistent with plant-soil feedback (Table 1). Above and belowground biomass responded similarly to inoculation treatments (data not presented) and thus, we only report total biomass responses below. The survival of *E. obliqua* seedlings did not significantly vary in response to inoculation treatments, while the total biomass of surviving seedlings was influenced by a significant interaction ($p = 0.025$) between tree species and fire. However, there were no significant pair-wise differences among inoculation treatments following Tukey-Kramer pair-wise tests ($p > 0.05$). The root:shoot ratio of *E. obliqua* seedlings was not significantly influenced by any soil inoculation treatment. Similar to *E. obliqua*, the survival of *E. globulus* seedlings did not significantly vary in response to inoculation treatments. However, the total biomass of the surviving *E. globulus* seedlings was significantly influenced ($p = 0.002$) by an interaction between tree species and fire. This interaction was driven by two significant pair-wise differences among inoculation treatments. Specifically, total biomass was two-fold greater when inoculated with *E. globulus* as opposed to *E. obliqua* soils from unburnt forest patches (Tukey-Kramer, $p = 0.036$), indicating a positive plant-soil feedback. However, this effect was absent when *E. globulus* seedlings were inoculated with soils from burnt stands. Further, the total biomass of *E. globulus* seedlings was more than two-fold greater when grown in potting soil inoculated with unburnt *E. globulus* as opposed to burnt *E. globulus* soils (Tukey-Kramer, $p = 0.008$).

Table 1. Results of mixed linear models analysing for the influence of soil inoculation treatment on the survival and biomass traits of *E. globulus* or *E. obliqua* seedlings. For each test, the numerator and denominator degrees of freedom as well as the F and P value are reported for each variable and each species separately. Bold values indicate statistical significance at $\alpha < 0.05$.

	Tree species		**Burning**		**Tree species x Burning**	
	$F_{(1,53-68)}$	**P**	**$F_{(1,53-68)}$**	**P**	**$F_{(1,53-68)}$**	**P**
E. obliqua						
Survival	0.0	0.961	0.4	0.527	2.4	0.126
Total biomass	0.3	0.614	0.3	0.572	5.3	**0.025**
Root to shoot ratio	0.8	0.383	0.4	0.552	1.7	0.195
E. globulus						
Survival	0.3	0.565	0.0	0.853	1.0	0.311
Total biomass	0.5	0.469	2.4	0.125	10.2	**0.002**
Root to shoot ratio	0.2	0.640	2.1	0.153	0.1	0.823

In support of the second hypothesis, plant-soil feedbacks differed when seedlings were inoculated with burnt versus unburnt soils (Figure 1). Although, only the positive feedback of *E. globulus* inoculated with soils collected from unburnt stands was statistically significant, the sign of plant-soil feedbacks was reversed in the burnt stands. For instance, *E. globulus* displayed a positive feedback when inoculated with soils collected from unburnt stands, but a trend for a negative feedback when inoculated with soils collected from burnt stands. In contrast, *E. obliqua* displayed a trend for a negative feedback when inoculated with soil collected from unburnt stands, but a trend for a positive feedback when inoculated with soils collected from burnt stands.

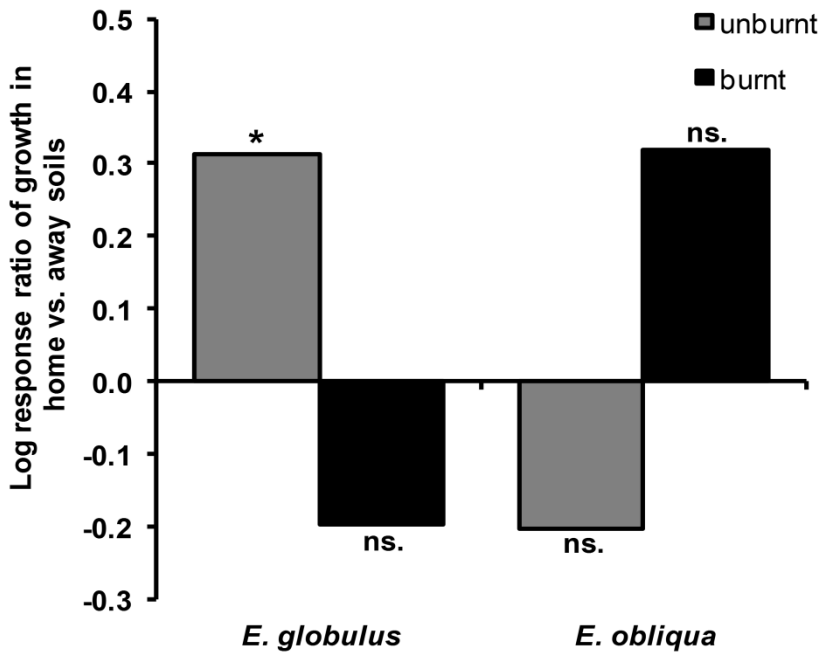

Figure 1. Log response ratios of each species inoculated with home versus away soils collected from either unburnt or burnt forest stands. Response ratios are calculated from least squares means obtained from mixed linear models analysing for the effects of tree species, forest fire and their interaction on the total biomass of each species individually. The star above the *E. globulus* unburnt response ratio indicates a significant difference (pair-wise Tukey-Kramer comparison; $t_{62} = 3.0$, $p = 0.021$) between the total biomass of *E. globulus* seedlings inoculated with *E. globulus* (home) and *E. obliqua* (away) soils collected from unburnt stands.

Discussion

Overall, our data suggest that eucalypt species are sensitive to local variation in soil biota. Seedling responses to inoculum were likely driven by soil biota, as we only introduced a very small quantity of field soils to forestry tubes, thus any influence of soil chemical properties are unlikely (Brinkman et al. 2010; Kulmatiski and Kardol 2008). While previous studies have investigated the growth of eucalypt seedlings in response to variation in whole field soils (Chen et al. 2006; Harvest et al. 2008) or individual microbial isolates (e.g. Chen et al. 2006; Lu et al. 1998), we are aware of only a single study that has taken an inoculum-based approach to investigate the

growth responses of eucalypt seedlings to spatial variation in soil communities. In this case, Ellis and Pennington (1992) found that the inhibitory effects of soils collected from a dieback and grassland site on the growth of *Eucalyptus delegatensis* seedlings was overcome by the addition of soil inoculum sourced from a healthy eucalypt forest, indicating variation in soil biota among localities. We show that eucalypts are also responsive to variation in soil communties on a much smaller scale and that these reponses are species-specific. These findings suggest variation in soil communities may affect the performance of the juvenile phases of eucalypt species at establishment differentially, which could impact their ecological dynamics (i.e., competitive interactions).

Local variation in soil biota appeared to be associated with tree species and patterns of forest fire within our study site. Although an observed effect of tree species on soil communities under field conditions may just reflect variation in the microhabitat occupied by the tree species, the influence of tree species on soil communities in our study could represent soil conditioning effects (Lejon et al. 2005; Maron et al. 2014; Trocha et al. 2012). Few studies have investigated interspecific variation in eucalypt soil conditioning (Anderson et al. 2013; Orozco-Aceves et al. 2015; Sayad et al. 2012). However, these studies show that eucalypt species may differentially condition both soil chemical and biotic characteristics under both glasshouse and field conditions. For example, Anderson et al. (2013) found that fungal community composition differed between *E. saligna* and *E. sideroxylon* soils after approximately 5 months of conditioning in a greenhouse experiment. The authors postulated that greater quanties of carbon supplied to the rhizosphere or more rapid depletion of water by *E. saligna* may have driven these differeces. At the same time, our results suggest that fire influenced soil communities, with performance consequences for eucalypt seedlings. A well documented consequence of fire in eucalypt forest is the 'ashbed effect' (Humphreys and Lambert 1965; Loneragan and Loneragan 1964; Pryor 1963), where the germination and performance of eucalypt seedlings is enhanced following fire, particularly seedlings of species belonging to the ash group (subgenus *Eucalyptus*, series *Obliquae*) (e.g., Ashton and Attiwill 1994; Neyland et al. 2009), which includes *E. obliqua*. The ashbed effect is thought to be mainly driven by fire-induced changes to soil physiochemical properties, but also may be in part driven by the sterilisation of antagonistic soil microorganisms (Keeley and

Fotheringham 2000). While we found no responses consistent with the sterilisation of antagonistic soil microorganisms, our findings did suggest that fire may have sterilised beneficial microorganisms. For example, *E. globulus* seedlings performed better when inoculated with unburnt as opposed to burnt *E. globulus* soils. Indeed, fire can influence both arbuscular mycorrhizal fungi and bacterial community composition, with effects lasting at least a year (Xiang et al. 2015; Xiang et al. 2014). Although we found no evidence for a microbial component to the ashbed effect, our results argue that the influence of fire on soil microbes, and subsequently plant growth, should be considered as a consequence of forest fire.

We suggest that external factors, in this case forest fire, may influence plant-soil feedback. While previous studies have shown that external abiotic factors can influence plant-soil feedback (Manning et al. 2008; Schradin and Cipollini 2012), to our knowledge, we are the first to investigate the influence of forest fire. We observed responses consistent with a positive plant-soil feedback in *E. globulus*, where seedling performance was significantly enhanced when inoculated with *E. globulus* as opposed to *E. obliqua* soils. This response was likely driven by beneficial organisms within inoculum collected from beneath mature *E. globulus* trees, as seedlings inoculated with unburnt *E. globulus* soils performed substantially better than seedlings inoculated with burnt *E. globulus* soils. In contrast, *E. obliqua* seedlings displayed a negative, but insignificant plant-soil feedback. We are only aware of a single study that has analysed for plant-soil feedback in *Eucalyptus*, where Orozco-Aceves et al. (2015) grew *E. marginata* seedlings in field soils conditioned by *Pinus radiata* and *E. saligna* (away soils) as well as *E. marginata* (home soils) trees. However, despite significant soil conditioning effects by the studied species, *E. marginata* displayed no significant feedback. This indicates that the presence of plant-soil feedback may vary among eucalypt species, possibly contributing to differences in their competitive interactions. For instance, within mixed stands of *E. globulus* and *E. obliqua*, the positive feedback of *E. globulus* may translate to a competitive advantage over *E. obliqua*. However, we found that this positive feedback was absent when *E. globulus* was inoculated with soils collected from burnt stands, indicating that fire may disrupt plant-soil feedbacks. As most seedling recruitment in eucalypt forest occurs following fire (Gill 1997), this positive feedback effect may not be important during the early establishment of *E. globulus*

seedlings in the wild, but could be during later growth. These findings suggest that, through changes to soil microbial communities, both soil conditioning and fire may influence plant performance. Therefore, in forest communities that experience regular fires, plant-soil feedbacks should not be considered independently as drivers of plant community structure and dynamics.

Conclusions

We show that soil communities may vary at a local scale within native eucalypt forest and this variation is associated with both differing eucalypt species and patterns of forest fire. However, further research will be required to confirm that variation in soil communities among eucalypt species in the field is a result of soil conditioning. Further, this variation in soil biota can in turn lead to species-specific feedback effects, which may affect the competitive interactions of species at establishment with lasting consequences for community structure. Lastly, the results of this study suggest that forest fire may disrupt plant-soil feedbacks. These findings argue that plant-soil feedback and forest disturbances should not be considered independently as drivers of plant community structure and dynamics, particularly with disturbance events such as fire predicted to become more frequent with global change (McDowell et al. 2015).

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Chapter 3
Phylogenetic relatedness can predict plant-soil feedbacks

This chapter is prepared for submission.

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Summary

Plant-soil feedbacks play important roles in maintaining biodiversity and the structure of complex forest communities, with species varying in the way they both condition and respond to conditioned soils. A well-documented response to soil conditioning is the Janzen-Connell effect, whereby species perform poorly in conspecific compared to heterospecific soils due to the accumulation of host-specific soil pathogens. This adverse effect has recently been extended to phylogenetically related species, termed the 'phylogenetic Janzen-Connell effect', but the generality of this effect remains unclear.

Using 14 native eucalypt species representing the two subgenera (*Eucalyptus* and *Symphyomyrtus*), we tested the generality of the phylogenetic Janzen-Connell effect. To do this we conducted a fully factorial glasshouse experiment testing the performance of seedlings of each focal *Eucalyptus* species in soils inoculated with soils previously conditioned by each species.

For each focal species, feedback was calculated as the slope of the linear regression of its relative response to each conditioned soil against its phylogenetic distance from the soil conditioning species. Responses studied were relative survival, growth rate, aboveground, belowground and total biomass, as well as the root to shoot ratio. The response slopes for each species were mapped onto a molecular phylogeny and tested for a phylogenetic signal.

We detected a range of responses to increasing phylogenetic distance between focal and soil conditioning species, with most showing positive or neutral responses. Focal species also exhibited a significant phylogenetic signal in their responses to increasing phylogenetic distance to soil conditioning species, which appeared to be mainly driven by subgeneric differences. Species from subgenus *Eucalyptus* performed better when inoculated with soils conditioned by more distant relatives (i.e., displayed a phylogenetic Janzen-Connell effect), while species from subgenus *Symphyomyrtus* either showed neutral or small negative responses.

Our results argue that phylogenetic Janzen-Connell effects do occur, but are dependent on the phylogenetic lineage and past evolutionary history of the focal species which may contribute to patterns of coexistence of these species in the field.

Introduction

Plants affect soil properties or 'condition' soils through the addition of chemical compounds and organic matter, influencing water and nutrient availability as well as providing habitat and resources for microorganisms (Ehrenfeld et al. 2005). Soil conditioning by plants may in turn have performance consequences for the individual, conspecifics or heterospecifics via plant-soil feedbacks (van der Putten et al. 2013). Plant-soil feedbacks have important landscape-level consequences for plant coexistence, diversity and abundance in temperate and tropical ecosystems (Johnson et al. 2012; McCarthy-Neumann and Kobe 2010a; b). For example, negative plant-soil feedbacks have been identified as a dominant force structuring patterns of diversity in tropical ecosystems (Liu et al. 2012; Mangan et al. 2010; Terborgh 2012). In these cases the accumulation of host specific soil pathogens in proximity to adult trees reduces the performance of conspecific seedlings, while allowing relatively unhindered performance of heterospecific seedlings (Mangan et al. 2010). This phenomenon is known as the Janzen-Connell effect (Connell 1971; Janzen 1970), where parental trees accumulate host specific enemies such as, herbivores as well as foliar and soil pathogens that inhibit the survival of offspring (Bagchi et al. 2014; Mangan et al. 2010; Terborgh 2012). Further, there is some evidence to suggest that this effect may be influenced by phylogenetic relatedness between focal and soil species thereby producing a 'phylogenetic Janzen-Connell effect' (Liu et al. 2012). In this case, the accumulation of fungal pathogens in the soils of mature trees reduced the survival of conspecific seedlings and even closely related species. However, in a recent review, Mehrabi and Tuck (2015) found no evidence to suggest that plant-soil feedbacks were associated with phylogenetic relatedness between focal and soil species. An explanation for this might be that, depending on evolutionary history, different focal species exhibit different feedback patterns with increasing phylogenetic distance to conditioning species.

Shared evolutionary history has been shown to influence a wide range of plant interactions including herbivore damage, community assemblage and diversity (Hill and Kotanen 2011; Nakadai et al. 2014; Watanabe et al. 2014), fungal pathogen susceptibility (Gilbert and Webb 2007) and ectomycorrhizal fungal species richness and community assembly (Tedersoo et al. 2013). These effects can occur through phylogenetic niche conservatism (Harvey and Pagel 1991), where ancestral traits are

conserved in groups of closely related species as a result of various evolutionary processes (Crisp and Cook 2012; Losos 2008). As such, plant phylogeny can also be a valuable tool for understanding plant-soil linkages and feedbacks. Indeed, a recent study has identified a phylogenetic effect on plant-soil feedbacks, where the direction and magnitude of seedling growth responses to soils conditioned by conspecifics compared to heterospecifics was specific to phylogenetic lineages (Anacker et al. 2014). Thus, susceptibility to a phylogenetic Janzen-Connell effect may also be unique to certain plant lineages.

Australian trees of the genus *Eucalyptus* represent an ideal system to investigate the role of evolutionary history on plant-soil linkages and feedbacks. On the island of Tasmania, Australia, eucalypt species typically co-exist in mixed stands that tend to include at least one species from each of the two larger eucalypt subgenera (subg.), *Eucalyptus* (formerly known as *Monocalyptus*) and *Symphyomyrtus* (Austin et al. 1983; Davidson and Reid 1980; Duff et al. 1983). Many key ecological differences between the subgenera are suggested to maintain this coexistence, including differences in germination, growth, biomass allocation and susceptibility to mammalian herbivores, insect pests and fungal pathogens (Eschler et al. 2000; Noble 1989; Stone et al. 1998; Wallis et al. 2010). There is also evidence that the subgenera differ in their relationships with soil pathogens and mycorrhizae (Noble 1989; Podger and Batini 1971), suggesting that phylogenetic differences in plant-soil feedbacks should also be considered as a potential driver of stand dynamics. Utilising 14 eucalypt species representing the subgenera *Eucalyptus* and *Symphyomyrtus*, we conducted a fully factorial experiment assessing the survival and growth responses of each species grown in potting soil inoculated with soils previously conditioned by each species. We used an inoculum-based method to exclude the influence of conditioning effects on soil chemical properties (Mangan et al. 2010; Maron et al. 2014; van Grunsven et al. 2007). Specifically, we examined whether (i) species' performances varied in response to increasing phylogenetic distance to soil conditioning species and (ii) whether these responses could also be explained by plant evolutionary history.

Materials and methods

Soil conditioning

In order to conduct the plant-soil feedback experiment, conditioned soils were required to inoculate seedlings. Open-pollinated seed of 14 native Tasmanian eucalypt species was obtained from Forestry Tasmania (http://www.forestrytas.com.au/), wet stratified and germinated as in Senior et al. (2013). Five species were selected from subg. *Eucalyptus* and nine species from subg. *Symphyomyrtus* (see Table 1). Twelve seedlings of each species were randomly selected from germination trays and grown within forestry tubes (200 ml) filled with non-sterile commercial potting mix consisting of eight parts composted pine bark and three parts coarse river sand with added macro- and micronutrients from Osmocote® For Natives low phosphorus, slow-release fertiliser (Scotts® Australia Pty Ltd, Baulkham Hills, NSW, Australia); this potting mix was consistently used throughout all aspects of the experiment. The potting mix included approximately 1.92, 0.16, 1.09 g Kg^{-1} dry mass of nitrogen (N), phosphorus (P) and potassium (K), respectively, that was slow-released over approximately six months. No extra fertiliser was applied during any part of the experiment. After a period of six months, plants of each species were planted in six evenly spaced positions within each of two replicate 33 L pots. The pots were then placed within a completely randomised common garden experiment that was located adjacent to a patch of native eucalypt forest dominated by *E. pulchella* (subgenus *Eucalyptus*), *E. globulus* and *E. viminalis* (subgenus *Symphyomyrtus*), where pots were subsequently exposed to colonisation by local soil biota. Plants were grown for a further two years in this outdoor location to differentially condition soils. Inoculum was then obtained from both replicate pots of each species and kept separate.

Plant-soil feedback experiment

To determine if differential conditioning by eucalypt species could impact seedlings of the next generation we tested for the effects of conditioned soils on seedling growth with a plant-soil feedback experiment. Eucalypt seedlings were grown from the same seed lots as those used in the conditioning experiment. Before sowing, the seed of each focal species was wet stratified to enhance germination as in Senior et al. (2013). Seed was then sprinkled over trays containing vermiculite and grown for

approximately 5 weeks until individuals of each focal species had developed their first pair of true leaves.

In order to test for feedback effects, seedlings from each of the 14 focal species were grown in potting mix inoculated with each previously conditioned soil in a fully factorial design. The design consisted of 28 soils (14 conditioning species x two replicate pots) and 14 focal species by three seedlings of each focal species (which were ultimately averaged to provide better estimates of the effects of inocula on each species), totalling to 1,176 seedlings. For each of the 28 conditioned soils, forty-two forestry tubes (14 focal species x 3 seedlings) were filled three quarters full with commercial potting mix. The two year old soil conditioning pots were destructively harvested at random over a period of one week. The above- and belowground biomass was removed and the soil of each pot was sieved through a 1 cm^2 mesh so that small root fragments could pass, such that root-associated microbes were included in the soils. Each soil was then homogenised to create a uniform mixture for the inoculation of seedlings. Two teaspoons of inoculum soil was then spread over the soil surface of each of the 42 forestry tubes. The top quarter of each forestry tube was left empty to prevent cross-contamination of inoculum between tubes during watering. Three seedlings of each focal species were transplanted directly into the inoculum of each conditioning pot, each within a separate forestry tube. Forestry tubes were organised in a randomised complete block design consisting of three replicates using the software package CycdesignN (https://www.vsni.co.uk/software/cycdesign/), with each conditioned soil by focal species combination represented once in each block. During the following week dead seedlings were replaced. Thereafter, replacement ceased, as deaths may have been due to treatment effects. Three weeks after this point and every three weeks thereafter, the height of seedlings (cm) was recorded. Height measurements were converted to growth rates using the slope of the regression of age and height.

After approximately four months of growth, the surviving seedlings were destructively harvested to test for feedback effects. Seedlings were carefully removed from their forestry tubes, with soil gently shaken and massaged off the roots. The roots were rinsed to wash off any remaining soil. Seedlings were cut at the root collar to yield above- and belowground biomass. The above- and belowground plant parts were placed in separate paper bags, dried at 60 ℃ for 48 hours and then

weighed. The belowground biomass was divided by aboveground biomass to yield root to shoot ratio and both above- and belowground biomass were summed to yield total biomass.

Phylogenetic analysis

We constructed a phylogeny of the 14 eucalypt species used in this experiment using Diversity Arrays Technology (DArT) markers, which have previously been used to resolve species-level phylogenetic relationships in eucalypts (Steane et al. 2011). We used presence/absence data from a set of 3,885 DArT markers for three individuals of each of 27 eucalypt species (26 are native to Tasmania and one, *E. nitens*, is closely related but not native to Tasmania). The Tasmanian species included samples from the same seedlots as used in the pot experiment. A species-level (consensus) dataset was compiled by Diversity Arrays Technology Pty. Ltd., Yarralumla, ACT, Australia (http://www.diversityarrays.com). Specifically, for each marker, a given species was given a 0 if all three individuals of that species scored 0, a 1 if all three individuals of that species scored a 1, or treated as a missing value if polymorphic across individuals. Given that the data were binary, they were analysed as discrete character data (Lewis 2001) using the program Bayesian Evolutionary Analysis by Sampling Trees (BEAST version1.75; (Drummond and Rambaut 2007). In BEAST are included BEAUti, LogCombiner, and TreeAnnotator software, the use for which we describe here. We ran 4 chains totalling 100,000,000 steps each, with sampling every 1000th step and estimating the rate of evolution from a normal distribution around 1 (specified using BEAUti). The root of the consensus phylogeny was assigned an arbitrary age of one. All chains reached convergence on the same posterior distribution and were combined into a single tree file using LogCombiner. From these trees we developed a consensus phylogeny by removing the first 100,000 (25%) sampled trees as burn-in, identified those with the best-supported topologies, and calculating posterior probabilities at each node using TreeAnnotator. We pruned the consensus phylogeny to include only the species used in this analysis; in this phylogeny all nodes had bootstrap values greater than 60%.

Statistical analysis

We examined whether phylogenetic distance between focal and soil conditioning species influenced the performance of the focal species across soils (i.e., plant-soil

feedbacks). We calculated individual plant-soil feedbacks as the log-transformed ratio of response (Hedges et al. 1999) for six traits: survival, growth rate, aboveground biomass, belowground biomass, total biomass and root to shoot ratio. Specifically, for each focal species, we took the logarithm of the average trait value of seedlings inoculated with each of the 14 soils (averaging across both conditioning pots) divided by the average trait value of seedlings inoculated with their own conditioned soils (averaging across both conditioning pots). Feedbacks are often calculated as the logarithm of a species' growth within its own soil divided by growth in a heterospecific soil (Brinkman et al. 2010; Petermann et al. 2008). However, we took the logarithm of each species' trait value in a heterospecific soil divided by its trait value in its own soil, so that responses consistent with a phylogenetic Janzen-Connell effect were indicated by a positive relationship between the relative performance of a focal species and increasing phylogenetic distance between itself and conditioning species. We calculated a total of 14 response ratios for each of the 14 focal species and for each of the six traits. Variation in the depth of potting soil within forestry tubes, resulting from variation among planters (e.g., how firmly soil was pressed around seedlings), had a significant effect on seedling survival and growth traits (data not shown). We accounted for this effect by using the residuals of each seedling trait regressed against soil depths in our calculations of response ratios. For each focal species, we then regressed the log transformed response ratios of each trait against phylogenetic distance between focal and soil conditioning species (ranging from 0.08 between *E. cordata* and *E. urnigera* up to 1.99 between *E. amygdalina* and *E. dalrympleana*) to obtain the slope (β) and test for the significance of each relationship. *Eucalyptus perriniana* was removed from this analysis due to high mortality, and thus low sample size, during the experiment, leaving 13 focal species. All regressions were implemented as linear models in the statistical package R (R Development Core Team 2014) using the package *stats*. Under visual inspection of the regressions, the subgenera tended to form two distinct data clouds, given the deep basal split in the phylogeny. However, the slope of a linear model was the best metric to show the response of each species to increasing phylogenetic distance to soil conditioning species.

To determine whether the direction and magnitude of relationships between the relative performance of eucalypt species and phylogenetic distance between focal and conditioning species were influenced by shared evolutionary history, we mapped regression slopes (see above) for the survival and growth responses of each species onto the phylogeny and calculated Blomberg's K (Blomberg et al. 2003) using the function *phylosig* within the package *phytools* (Revell, 2012). This was a reasonable approach given that the phylogeny was ultrametric and well-balanced, and thus, the x-axes were similar across different species' regressions; this approach may not be suitable for non-balanced phylogenies. In our calculations of K, we incorporated the standard errors of the linear regression coefficients, which accounted for error in the accuracy of predictions (Ives et al. 2007). The K statistic is a measure of phylogenetic signal or the tendency of closely related species to resemble each other more than they resemble species drawn at random from a phylogenetic tree (Blomberg et al. 2003). Values less than 1 imply that close relatives resemble each other less than expected under Brownian motion evolution, while values greater than 1 indicate that closely related species are more similar than expected under this model. Phylogenetic signal in the relationships between the relative performance of eucalypt species and phylogenetic distance between focal and conditioning species was presented by mapping species regression slopes onto the eucalypt phylogeny using the function *plot.phylo* within the package *ape*.

Results

We found that more closely related species conditioned their soils in a manner that produced phylogenetic patterns in seedling survival and growth feedbacks across soils. Species generally responded differentially to inoculation with heterospecific versus conspecific conditioned soils, where all subgenus *Eucalyptus* species displayed significant positive responses, three of the eight *Symphyomyrtus* species generally displayed significant negative responses, one displayed mostly positive responses and one displayed variable responses, depending on the trait (see Appendix A, Table A1). Phylogenetic distance was a major driver of the magnitude of seedling responses to inoculation with heterospecific versus conspecific soils. Focal species displayed a range of responses to potting soils inoculated with soils conditioned by species of increasing phylogenetic distance (Table 1). Only three of the 13 focal species displayed significant relationships between survival responses

and phylogenetic distance to conditioning species. These relationships were all positive (i.e., negative plant-soil feedback), where the survival of seedlings increased with phylogenetic distance between focal and conditioning species. For instance, the survival response ratios of *E. obliqua* increased from <0.01 to 0.09 to 0.18 when inoculated with soils conditioned by its closest relative (*E. pauciflora*), a moderate relative (*E. amygdalina*) and a distant relative (*E. cordata*), respectively, compared to its own soils. For the surviving seedlings, six focal species displayed significant relationships between growth rate responses and phylogenetic distance to conditioning species, where one was negative (i.e., positive plant-soil feedback) and five were positive. For instance, the growth rate response ratios of *E. pauciflora* increased from <0.01 to 0.04 to 0.18 when inoculated with soils conditioned by its closest relative (*E. obliqua*), a moderate relative (*E. nitida*) and a distant relative (*E. dalrympleana*), respectively, compared to its own soils. In contrast, the growth rate response ratios of *E. rodwayi* decreased from -0.02 to -0.21 to -0.38 when inoculated with soils conditioned by its closest relative (*E. ovata*), a moderate relative (*E. globulus*) and a distant relative (*E. nitida*), respectively, compared to its own soils. For all biomass traits, four focal species displayed significant responses to increasing phylogenetic distance to conditioning species, all of which were positive. For instance, the total biomass response ratios of *E. nitida* increased from 0.04 to 0.05 to 0.37 when inoculated with soils conditioned by its closest relative (*E. amygdalina*), a moderate relative (*E. pauciflora*) and a distant relative (*E. globulus*), respectively, compared to its own soils. The root to shoot ratio responses of three species displayed significant relationships with increasing phylogenetic distance to soil conditioning species. These relationships were all positive, where the root to shoot ratio increased when inoculated with soils of increasing phylogenetic distance (i.e., plants allocated more biomass belowground in soils conditioned by more distant relatives). These findings show that phylogenetic distance between focal and conditioning species is important for the performance of seedlings, but the magnitude and direction of the relationship depends on the focal plant species.

Table 1. Species display variable responses to soils conditioned by species of increasing phylogenetic distance. The number of surviving seedlings of each species at the conclusion of the experiment (n) as well as the slopes (β) and standard error (SE) of the linear relationships between the relative performance of each eucalypt species and phylogenetic distance between focal and conditioning species. Bold values represent significance, where * P = < 0.05, ** P = < 0.01 and *** P = < 0.001.

			Variable											
			Survival		**Growth rate**		**Aboveground biomass**		**Belowground biomass**		**Total biomass**		**Root to shoot ratio**	
Subgenus	**Species**	**n**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**
Eucalyptus	***E. obliqua***	66	**0.06***	0.02	**0.06****	0.02	**0.12****	0.04	**0.12***	0.04	**0.12****	0.04	**0.07***	0.03
	E. pauciflora	69	0.05	0.03	**0.06*****	0.01	**0.10****	0.03	**0.09****	0.02	**0.10****	0.02	0.01	0.03
	E. nitida	67	**0.07****	0.02	**0.06****	0.02	**0.13*****	0.02	**0.14*****	0.03	**0.13*****	0.02	**0.05***	0.02
	E. radiata	63	0.05	0.02	**0.06***	0.02	**0.08***	0.03	**0.09***	0.04	**0.08***	0.03	0.01	0.02
	E. amygdalina	64	**0.10****	0.03	**0.06***	0.03	0.06	0.04	0.07	0.04	0.06	0.04	0.01	0.03
Symphyomyrtus	***E. rodwayi***	54	-<0.01	0.05	**-0.05***	0.02	-0.06	0.04	-0.07	0.04	-0.06	0.04	-0.02	0.04
	E. ovata	52	-0.01	0.04	-0.04	0.02	-0.05	0.05	-0.05	0.05	-0.05	0.05	0.04	0.02
	E. barberi	56	<0.01	0.08	-0.06	0.04	-0.08	0.05	-0.09	0.06	-0.09	0.06	-0.02	0.03
	E. urnigera	57	-0.03	0.04	-0.03	0.02	-0.07	0.03	-0.07	0.04	-0.07	0.03	-0.02	0.04
	E. cordata	62	-0.01	0.03	-0.03	0.04	-0.06	0.06	-0.07	0.06	-0.07	0.06	-0.03	0.03
	E. subcrenulata	55	0.04	0.03	0.01	0.02	0.02	0.03	0.03	0.04	0.02	0.03	**0.08***	0.04
	E. globulus	71	0.04	0.03	0.02	0.03	0.02	0.03	0.01	0.03	0.02	0.03	-0.03	0.03
	E. dalrympleana	68	0.04	0.03	-0.03	0.04	-0.05	0.05	-0.07	0.06	-0.05	0.05	-0.05	0.04

We identified strong phylogenetic signals in the relationships between the responses of focal species and increasing phylogenetic distance to conditioning species (Table 2). Survival responses were phylogenetically conserved ($P < 0.01$), as were all biomass responses for which we calculated K values that were approximately 2. That is, focal species responded more similarly to soils conditioned by species of increasing phylogenetic distance within genetic lineages than focal species across genetic lineages. These phylogenetic signals were mainly driven by differences between subgenera (Fig. 1). Subgenus *Eucalyptus* species uniformly displayed strong positive relationships between seedling performance and increasing phylogenetic distance to soil conditioning species (i.e., negative plant-soil feedbacks). In contrast, subgenus *Symphyomyrtus* species displayed no relationship, or at most, relatively small positive or negative relationships.

Table 2. Species display phylogenetic signal in responses to soils conditioned by species of increasing phylogenetic distance. Tests of phylogenetic signal (Bloomberg's K) in the slopes of linear relationships between the relative performance of each eucalypt species and phylogenetic distance between focal and conditioning species.

Variable	K	P
Survival	1.2	0.002
Growth rate	2.3	0.001
Aboveground biomass	2.0	0.001
Belowground biomass	1.9	0.001
Total biomass	1.9	0.001
Root to shoot ratio	1.0	0.184

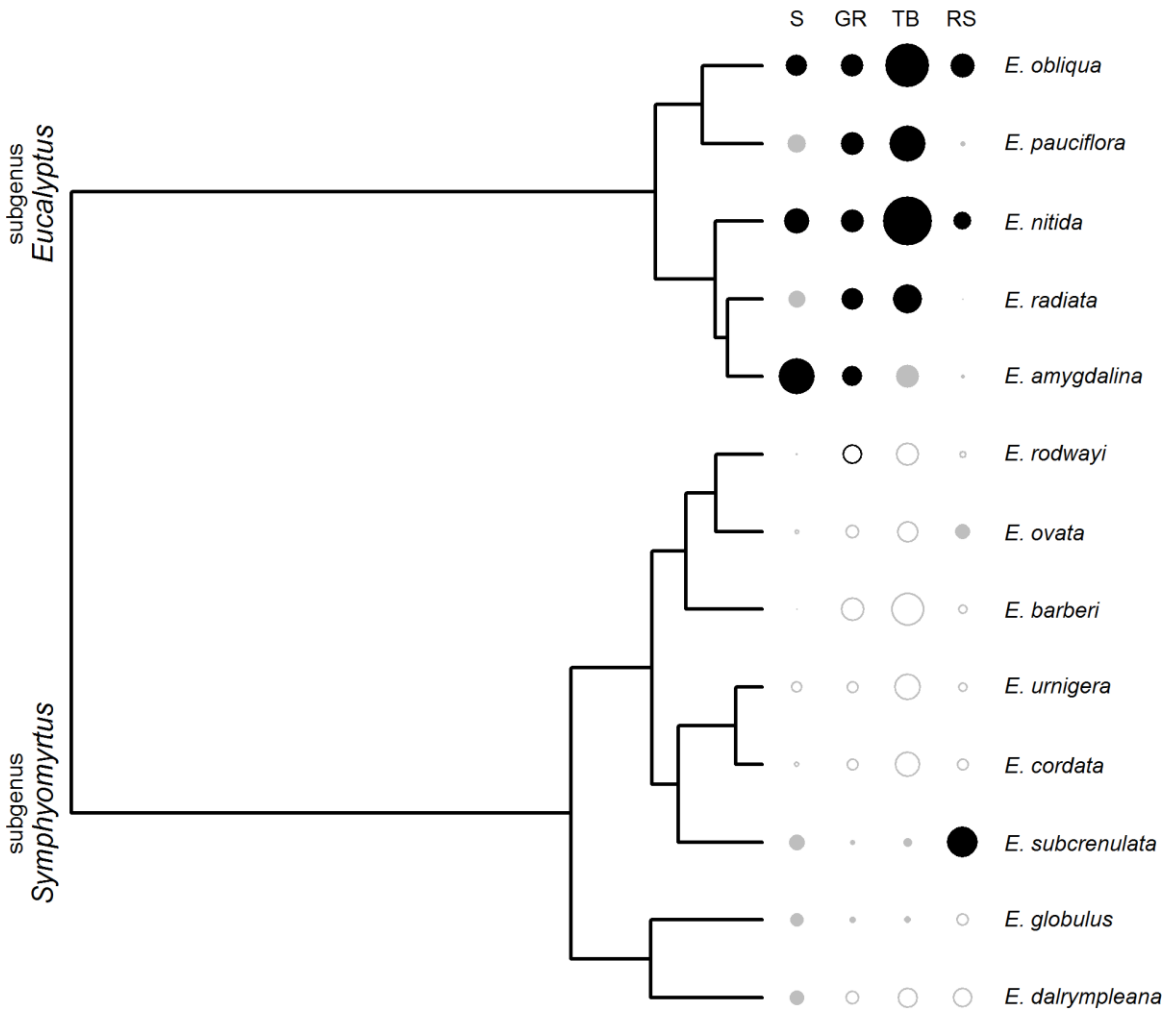

Figure 1. Phylogenetic lineages differ in responses to soils conditioned by species of increasing phylogenetic distance. Phylogeny of the eucalypt species (subgenus *Eucalyptu*s and *Symphyomyrtus*) used in the present study with mapped slopes of linear relationships between the relative survival (S), growth rate (GR), total biomass (TB) and root to shoot ratio (RS) of each eucalypt species and phylogenetic distance between focal and conditioning species. Above and belowground biomass relationships are not shown as they exhibited similar trends to total biomass. Full circles represent positive relationships between the responses of species to conditioned soils and increasing phylogenetic distance to soil conditioning species (i.e., a phylogenetic Janzen-Connell effect), while open circles represent negative relationships. The size of circles represents the strength of the relationships, with larger circles representing stronger relationships and black and grey coloured circles represent significant and non-significant responses, respectively.

Discussion

We provide clear evidence that plant species differentially condition their soils as shown by the subsequent growth of seedlings in potting soil inoculated with conditioned soils. Such soil conditioning is traditionally associated with either changes to soil biota or nutrient depletion (Anacker et al. 2014; Meisner et al. 2011; Perkins and Nowak 2012). In the present study, the use of small amounts of conditioned soils to inoculate the new potting soil indicates that the detected conditioning was likely driven largely by changes in soil biota. Many other studies have employed similar inoculation procedures and detected conditioning effects (Mangan et al. 2010; McCarthy-Neumann and Kobe 2010a; b), where growth responses to inoculation with soils conditioned by conspecifics or close relatives are often negative, as reported here. Fungicide treatments suggest that these effects are a consequence of the accumulation of host-specific fungal pathogens (Liu et al. 2012; Reinhart et al. 2003). Soil conditioning has been frequently demonstrated using field collected soils (Liu et al. 2012; Mangan et al. 2010; Pregitzer et al. 2010; Reinhart et al. 2003) and experimentally conditioned soils (Anacker et al. 2014; McCarthy-Neumann and Kobe 2010b; Perkins and Nowak 2012). While conditioning effects detected in field collected soils reflect soil conditioning by the focal species, these effects may be confounded by shared soil niches of tree species and soil organisms or variable tree age (i.e., timeframe of soil conditioning). By growing same-age tree species in a uniform potting medium for two years, we were able to demonstrate significant conditioning effects without such confounding factors.

We also show that the responses of focal tree species to soil conditioning can be dependent on the degree of phylogenetic relatedness between focal and conditioning species. We identified variable trends between seedling responses to conditioned soils and increasing phylogenetic distance between focal and conditioning species, where feedbacks ranged from positive (i.e., negative plant-soil feedback), to insignificant (i.e., neutral plant-soil feedback) to negative (i.e., positive plant-soil feedback). Half of the focal species showed significant relationships between performance on an inoculated soil and phylogenetic distance to conditioning species. This is contrary to recent studies reporting little to no effect of phylogenetic distance on plant-soil feedbacks (Mehrabi et al. 2015; Mehrabi and Tuck 2015). For example, a recent meta-regression of 329 experimental plant-soil feedback effects

demonstrated that the growth of species in conditioned soils was poorly predicted by phylogenetic distance between focal and soil conditioning species (Mehrabi and Tuck 2015). In this case, feedbacks were pooled across diverse groups of plant species, which may have masked interspecific variation in responses. Our results suggest that this could arise with trends among species that vary from positive to negative that when pooled bring about an overall neutral effect of phylogenetic relatedness on plant-soil feedbacks, resulting in important interspecific responses being missed.

Further, we show that the majority of significant responses to increasing phylogenetic distance to conditioning species were positive, consistent with a phylogenetic Janzen-Connell effect (Liu et al. 2012). Recent research demonstrates that forest communities may be phylogenetically structured, where neighbouring tree species are less phylogenetically related than expected by chance (Liu et al. 2012; Zhu et al. 2015). Experimental evidence suggests that these patterns are driven by a phylogenetic Janzen-Connell effect, where the performance of seedlings in conditioned soils is dependent upon the degree of phylogenetic relatedness between focal and conditioning species. For example, Liu et al. (2012) found that the relative survival of eight sub-tropical tree species in native soils collected from beneath co-occurring *Castanopsis fissa*, increased with increasing phylogenetic distance of the focal species to *C. fissa*. While we tested this relationship differently, by varying the conditioning species as opposed to the focal species, we confirm that the adverse effects of species-specific soil conditioning may diminish with decreasing phylogenetic relatedness of focal seedlings to conditioning species. Further, by including multiple conditioned soils, we also show that more phylogenetically similar species may condition soils in a manner that leads to phylogenetic patterns in plant-soil feedbacks. However, we also found that some species either did not significantly respond or performed more poorly when inoculated with soils conditioned by more distant relatives. This suggests that not all species are susceptible to a phylogenetic Janzen-Connell effect, at least with regards to conditioned soil biota. It is, therefore, of interest to predict this interspecific variation in order to understand the mechanisms driving the co-existence of plant species in complex communities.

Lastly, our results suggest that susceptibility to the phylogenetic Janzen-Connell effect can be predicted to some extent, and this is from knowing the phylogenetic relationships of the focal species themselves. Our findings corroborate recent experimental evidence of a significant phylogenetic signal in whole-soil feedbacks (heterospecific versus con-specific soils) within a Canadian old field community containing 57 species (Anacker et al. 2014). However, the influence of phylogenetic relatedness between focal and conditioning species on plant-soil feedbacks was not taken into account in this case. To our knowledge, we are the first to show that phylogenetic signal in plant-soil feedbacks may occur within a single plant genus. We identified clear phylogenetic signals in the survival and growth responses of species to soils conditioned by species of increasing phylogenetic distance. The observed phylogenetic signals appear to be driven by subgeneric differences, where subg. *Eucalyptus* species all displayed trends consistent with the phylogenetic Janzen-Connell effect, while subg. *Symphyomyrtus* species typically did not differentially respond to conditioned soils. While the possibly that these results are driven by a positive conditioning effect of subg. *Symphyomyrtus* species (i.e., an accumulation of mutualists) cannot be dismissed, a negative conditioning effect of subg. *Eucalyptus* species is more consistent with the general literature, where the vast majority of feedbacks in temperate and tropical ecosystems are negative (van der Putten et al. 2013). Specifically, adverse effects of subg. *Eucalyptus* conditioned soils could be due to an accumulation of host-specific pathogens in the soils of pot grown trees (Liu et al. 2012; Mangan et al. 2010; Terborgh 2012). Indeed, there is evidence to suggest that species belonging to the subgenus *Eucalyptus* are more susceptible to soil fungal pathogens (i.e., *Phytophthora*; Noble 1989; Podger and Batini 1971). This would suggest that focal subg. *Eucalyptus* species may have escaped host-specific soil pathogens when grown in soils conditioned by subg. *Symphyomyrtus* species, producing a phylogenetic Janzen-Connell effect. Although our results are only based on conditioning and feedbacks observed in a nursery environment and many factors could affect their inference to natural systems (e.g., soil nutrient content, a full native soil community and seedling developmental stage), these results suggest the possibility that subgeneric differences in plant-soil feedbacks may contribute to the structure of mixed eucalypt stands containing species belonging to each subgenus (Austin et al. 1983; Davidson and Reid 1980; Duff et al. 1983). Seedlings in this study reflect the early stages of eucalypt growth

(seedling and sapling stages), where plants are particularly vulnerable and sensitive to soil conditions. Plant-soil feedbacks that develop during this crucial establishing phase could have important and lasting consequences for community structure. Specifically, subg. *Eucalyptus* species may escape host-specific soil pathogens by growing in the soils of subg. *Symphyomyrtus* species, allowing greater survival and growth. However, subg. *Symphyomyrtus* species were generally unaffected by soil conditioning species, suggesting that soil biota may not limit the growth of these species to the same degree. However, in this case other factors such as, insect herbivory (Stone et al. 1998), may limit the growth of subg. *Symphyomyrtus* species and contribute to the co-existence of both subgenera.

In summary, we establish experimentally that the phylogenetic relationships between focal and conditioning species can be an important factor influencing the direction and magnitude of plant-soil feedbacks, but not for all species. When these phylogenetic relationships did significantly affect plant-soil feedbacks, the observed trends were consistent with a phylogenetic Janzen-Connell effect and susceptibility to this effect itself displayed phylogenetic signal. These findings suggest that phylogenetic relationships may predict the performance of forest trees in the soils of co-occurring species. However, further research is critical for making accurate inferences to natural systems. In particular, the conditioned soil biota and the overall soil fertility within our pots likely differed to those found within native soils, which could influence the outcome of feedbacks observed in the present study (Schradin and Cipollini 2012). We suggest that future studies use reciprocal transplant experiments to determine whether patterns discovered here exist in the field. Continued investigation of phylogenetic structure in observations of plant-soil feedbacks holds the potential to increase our understanding of the mechanisms structuring plant communities and vegetation dynamics by uncovering the linkages between plant ecology and evolution.

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Chapter 4
Experimental evidence for phylogenetic signal in soil microbial community conditioning

This chapter is prepared for submission.

John K. Senior, Brad M. Potts, Andrew Bissett, Julianne M. O'Reilly-Wapstra, Joseph K. Bailey, Morag Glen, Jennifer A. Schweitzer. Experimental evidence for phylogenetic signal in soil microbial community conditioning.

Summary

Findings from our experimental system show that plant-soil feedbacks display a phylogenetic signal, where closely related plant species display similar feedbacks. These effects are often attributed to plant conditioning of soil microbes, yet explicit evidence for phylogenetic signal in the conditioning of soil microbial communities is lacking. In this study, we tested for differential soil microbial conditioning by two *Eucalyptus* subgenera, *Eucalyptus* and *Symphyomyrtus*.

Soils were conditioned by two replicates of five subgenus *Eucalyptus* species and nine subgenus *Symphyomyrtus* species grown within a common garden pot experiment over a period of two years. We extracted DNA from each conditioned soil and the bacterial and fungal community profiles of each pot was assessed through 454 pyrosequencing of the fungal internal transcribed spacer (ITS) region and bacterial 16S rRNA genes. Fungal and bacterial sequences were clustered into operational taxonomical units (OTUs) that were further aggregated to the family taxonomic level for both bacteria and fungi. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differential conditioning effects of each subgenus on the community composition of fungi and bacteria.

We found that subgenus had a significant effect on fungal, but not bacterial, community composition within conditioned soils at both the OTU and family level, indicating phylogenetic signal in the conditioning of fungal communities. Further, soils sampled from subgenus *Eucalyptus* species more frequently contained fungal taxa that exhibit pathogenic relationships with eucalypts. These findings provide evidence that closely related plant species condition fungal communities similarly, possibly leading to phylogenetic signal in plant-soil feedback.

Introduction

Soil microorganisms comprise a significant portion of Earth's biodiversity and are key regulators of ecosystem processes and plant performance. It has been estimated that one gram of soil may contain as many as 10^{10} - 10^{11} bacteria (Horner-Devine et al. 2003) and up to 200 million fungal hyphae (Leake et al. 2004). These organisms may have direct and indirect effects on plants, often forming intimate associations with their hosts, with positive or negative effects on plant performance (reviewed in (Van Der Heijden et al. 2008b). Free-living soil microbes are strong regulators of both carbon (C) and nitrogen (N) cycling, and thus, plant productivity. For instance, most soil N (96-98%) is contained within dead organic matter that is broken down by soil microbes into forms that are accessible to plants via the process of N mineralisation (Schimel and Bennett 2004). At the same time some free-living soil microbes can have negative impacts on plant growth by competing for vital nutrients or transforming N into more labile forms such as, nitrate, which is readily lost from the system via leeching (Van Der Heijden et al. 2008b). Further, specific groups of soil microbes may form more intimate associations with plants, with direct positive or negative effects on performance. For instance, mycorrhizal fungi are estimated to form symbiotic associations with the roots of approximately 80% of all terrestrial plant species (Smith and Read 1997), providing increased resistance to drought or disease as well as supplying a range of limiting nutrients in exchange for carbon. Alternatively, soil microbial communities may include pathogens such as *Phytophthora spp.*, *Fusarium spp.* and *Pythium spp.*, which have been shown to attack a range of forest trees including oaks, acacias and eucalypts (see Burdon et al. 2006). Thus, an understanding of the drivers of variation in soil microbial communities may be crucial to predicting plant performance.

Soil microbial communities can respond to a variety of abiotic factors over broader scales (see de Vries et al. 2012), but it has been suggested that plant identity can best explain local variation in soil microbial communities (Burns et al. 2015; Orwin et al. 2010). This may be a result of soil conditioning, where plants can influence soil microorganisms through both direct and indirect mechanisms. Direct conditioning effects can occur through the exudation of specific compounds with either positive or negative effects on target organisms (Baetz and Martinoia 2014; Ehrenfeld et al. 2005). For instance, in the presence of a pathogen, *Arabidopsis thalina* exudes malic

acid to attract a beneficial bacterium, *Bacillus subtilis* (Rudrappa et al. 2008). Alternatively, barley exudes phenylpropanoids from its roots as a rapid defence response to infection with the fungal pathogen *Fusarium graminearum* (Lanoue et al. 2010). Plants can also condition soil microbes indirectly through variation in growth phenology, water and nutrient use, as well as the quantity and quality of organic inputs to soils (Ehrenfeld et al. 2005). For example, plant species with fast or slow growth rates tend to condition distinct microbial communities resulting from different plant traits (i.e., litter quality) that are associated with growth strategy (Baxendale et al. 2014; Orwin et al. 2010). Plant species often vary in the manner by which they condition soil microbes (e.g., Lejon et al. 2005; Orwin et al. 2010; Trocha et al. 2012), resulting from genetic variation in functional traits (e.g., relative growth rate, foliar chemistry and specific leaf area). This variation in conditioning may in turn feed back to affect the performance of conspecifics or heterospecifics via plant-soil feedbacks (van der Putten et al. 2013). Thus, the ability to predict variable microbial conditioning among plant species may allow greater understanding of plant responses to the soils of co-occurring species.

Herein, we test for the influence of plant evolutionary history on microbial conditioning within the dominant Australian genus, *Eucalyptus*. In a previous study, we found significant phylogenetic signal in the plant-soil feedbacks of eucalypt species belonging to the subgenera *Eucalyptus* and *Symphyomyrtus* (Chapter 3). Phylogenetic signal was primarily driven by species belonging to subgenus *Eucalyptus* performing better when inoculated with subgenus *Symphyomyrtus* conditioned soils as opposed to their own (i.e., negative plant-soil feedbacks), while subgenus *Symphyomyrtus* species performing equally when inoculated with soils conditioned by either subgenus. As we used an inoculum-based method to exclude the influence of conditioning effects on soil chemical properties (Brinkman et al. 2010; Kulmatiski and Kardol 2008), soil microbes were implicated as the causal agent. It was hypothesised that the negative feedback exhibited by subgenus *Eucalyptus* species was the result of the build-up of microbial elements in their conditioned soils which had deleterious effects specific to seedlings of this subgenus. Thus, to provide further evidence that phylogenetic signal in microbial conditioning drove observed differences in plant-soil feedbacks, we tested whether species belonging to each subgenus differentially conditioned soil microbial communities

using samples collected from the same soil conditioning experiment. The bacterial and fungal community profile of conditioned soils was assessed through 454 pyrosequencing of the fungal internal transcribed spacer (ITS) region and bacterial 16S rRNA genes. We here test the specific hypotheses that (i) soils conditioned by species of each subgenus would differ in microbial abundance and richness, (ii) bacterial and/or fungal community composition and (iii) that conditioned microbes were associated with previously observed eucalypt plant-soil feedbacks.

Materials and methods

Soil conditioning experiment

We grew 14 eucalypt species within a common pot experiment to determine whether the eucalypt subgenera, *Eucalyptus* and *Symphyomyrtus*, differentially conditioned soil microbial communities. Open-pollinated seed of 15 native Tasmanian eucalypt species was obtained from Forestry Tasmania (http://www.forestrytas.com.au/), wet stratified and germinated as in Senior et al. (2013). Five species were selected from subg. *Eucalyptus* and nine species from subg. *Symphyomyrtus*. Twelve seedlings of each species were randomly selected from germination trays and grown within forestry tubes (200 ml) filled with non-sterile commercial potting mix consisting of eight parts composted pine bark and three parts coarse river sand with added macro- and micronutrients from Osmocote® For Natives low phosphorus, slow-release fertiliser (Scotts® Australia Pty Ltd, Baulkham Hills, NSW, Australia); this potting mix was consistently used throughout all aspects of the experiment. The potting mix included approximately 1.92, 0.16, 1.09 g Kg^{-1} dry mass of nitrogen (N), phosphorus (P) and potassium (K), respectively, that was slow-released over approximately six months. No extra fertiliser was applied during any part of the experiment. After a period of six months, plants of each species were planted in six evenly spaced positions within each of two replicate 33 L pots. The pots were then placed within a completely randomised common garden experiment that was located adjacent to a patch of native eucalypt forest dominated by *E. pulchella* (subgenus *Eucalyptus*), *E. globulus* and *E. viminalis* (subgenus *Symphyomyrtus*), where pots were subsequently exposed to colonisation by local soil biota. Plants were grown for a further two years in this outdoor location to differentially condition soils.

After the two-year conditioning phase, soils were sampled to determine whether the eucalypt subgenera differentially conditioned soils. Three soil cores (2x15 cm) were taken from each replicate pot and bulked. Soil samples were then sieved (2 mm) in order to homogenise and remove any root material. Both the corer and sieve were washed with detergent, rinsed with water and dried between each sample to limit cross contamination of conditioned soils. A portion of each soil sample was taken for NO_3^- and NH_4^+ quantification (see below) and another portion was taken for DNA extraction. Samples intended for NO_3^- and NH_4^+ assays were refrigerated at 4 °C for less than 48 h before quantification, while samples intended for DNA extraction were snap frozen in liquid nitrogen and stored at – 80 °C. Following soil sampling, the aboveground biomass of each pot was harvested, dried to constant weight at 60 °C for 72 hours and weighed. Aboveground biomass values were used to determine whether microbial conditioning was related to variation in plant biomass.

DNA extraction and Pyrosequencing

Soil DNA was extracted from each soil sample and purified using the MoBio, Inc. PowerSoil DNA isolation kit according to the manufacturer's instructions (MoBio, Inc., Solana, CA, USA). DNA extracts were sent to Molecular Research LP (Mr DNA^{TM}), Shallowwater, Texas, USA, for PCR amplification and sequencing. PCR amplification of the bacterial 16S rRNA genes was carried out using the 27F-519R primer pair and the amplification of the fungal ITS region was carried out using the ITS1-ITS4 primer pair. The 16S and ITS PCR products were then sequenced separately using the 454 FLX titanium platform (Roche, Branford, CT), accordingly to the company protocols. Raw data were provided in the form of standard flowgram format (sff). Sequence analyses were performed on 16S rRNA gene and the ITS region separately using MOTHUR version 1.22.0 (Schloss et al. 2009) following the adapted sequence quality-control pipeline analysis described in detail in Schloss et al. (2011), until chimeric sequences were removed. Remaining sequences were then clustered into Operational Taxomomic Units (OTU) of 97% sequence similarity using USEARCH (version 5) (Edgar 2010). Reads were then mapped to these OTUs (97%) using USEARCH to produce an OTU table. OTUs were classified against Green Genes (DeSantis et al. 2006) for 16S and UNITE (Kõljalg et al. 2005) for fungal ITS using the RDP classifier (Wang et al. 2007) as implemented in MOTHUR (probability = 60%). OTU abundance was calculated as the total number

of OTUs detected in a sample, while richness was calculated as the number of unique OTUs detected in a sample.

Soil NO_3^- and NH_4^+ assays

We quantified the NO_3^- and NH_4^+ content of conditioned soils in order to determine whether variable microbial conditioning between subgenera was related to variation in soil nutrient content. Eight grams of each soil sample was weighed into 50 ml centrifuge tubes and combined with 40 ml of 2M KCl solution. The centrifuge tubes were agitated for one hour on an orbital shaker and centrifuged at 4000 rpm for three minutes to precipitate particulate matter from the extracts. Five millilitres of each extract was then drawn with separate syringes and filtered through 25 mm nylon (0.45 μm pore size) syringe filters into collection containers. The NO_3^- and NH_4^+ content of extracts was quantified using a Smartchem discrete analyser (Westco Scientific Instruments Inc., Brookfield, CT, USA), using the Smartchem 200 methods 375-100E-1 and 210-200B for NO_3^- and NH_3^+, respectively.

Statistical analysis

Mixed linear models were fitted to the pot-level data in the statistical program R (R Development Core Team 2014) to analyse for the effects of subgenus on OTU abundance and richness, soil NO_3^- and NH_4^+ and aboveground biomass using the function lme within the package *nlme*. Models included subgenus as a fixed effect and the random term species within subgenus was used to test for subgenus effects. The anova function from the package *lmerTest* was used to conduct F tests on each model. To test for the significance of the random term species within subgenus, the anova function was used to conduct log-likelihood tests on models with (lme) or without (lm from the package *stats*) the random term species within subgenus. The function residplot from the package *predictmeans* was used to check residuals for homoscedasticity and normality. Nitrate was natural log transformed, but no other variable required transformation.

To determine whether the subgenera differentially influenced soil microbial community composition, multivariate analyses were performed using the software PRIMER v.6 with the PERMANOVA+ add-on (Plymouth Marine Laboratory, Plymouth, United Kingdom). Multivariate analyses of fungal and bacterial communities were conducted at the taxonomic levels of OTU and family. Both

fungal and bacterial community datasets were converted to presence/absence data to give all OTUs or families within each sample equal weighting. All models were performed on Bray-Curtis similarity matrices of pot-level data. To analyse the relationships between fungal or bacterial communities and the variables, NO_3^-, NH_4^+ and aboveground biomass, separate distance-based linear models (DISTLMs) were fitted for each covariate at both the OTU and family taxonomic levels. Permutational multivariate analysis of variance (PERMAVOVA) was used to test for the effect of subgenus on fungal and bacterial community composition at both OTU and family levels. Models included subgenus as a fixed effect and the random effect species within subgenus which was used to test for subgenus effects. Ammonium was fitted as a covariate in all models, as the NH_4^+ content of pots significantly influenced fungal and bacterial community composition (DISTLM, $p < 0.05$). Models were performed with 999 permutations using type 1 sums of squares, which accounted for variation due to the soil NH_4^+ before testing for subgenus effects. Significant effects of NH_4^+ and subgenus on community data were visualised by plotting against the two axes derived from canonical analyses of principal coordinates (CAP). This analysis used the Bray-Curtis similarity matrix derived from pot-level data and identified independent axes of variation in community composition that were aligned with variation in NH_4^+ or subgenera. The similarity percentages (SIMPER) function was used to identify key microbial families contributing to dissimilarity in fungal community composition between the subgenera, also using the Bray-Curtis similarity matrix derived from pot-level data.

Generalised mixed linear models were fitted in R to determine whether the presence of key microbial families significantly differed between soils conditioned by species of each subgenus using the function glmer within the package *lme4*. These models included subgenus as a fixed effect and the random effect species within subgenus which was used to test for subgenus effects. Models were implemented using a binomial error distribution with a logit link function and included the covariate NH_4^+ under type three sums of squares.

Non-parametric Kendall rank correlations were fitted in R to test for significant relationships between key microbial families and plant-soil feedbacks using the function Kendall from the package *Kendall*. We compiled a species-level dataset of the proportion of pots belonging to each species in which key microbial families

were detected. Specifically, if a given microbial family was detected in both pots of a species it was scored a 1, if detected in a single pot it was scored 0.5 and if the family was not detected in either pot of a species it was scored a 0. Plant-soil feedback data was obtained from Chapter 3. In brief, feedback values were slopes (β) obtained from linear regressions between the performance of each species when inoculated with each of the conditioned soils compared with its own (log response ratios; Hedges et al. 1999) and the phylogenetic distance between itself and conditioning species. Positive slopes indicate that species perform better when inoculated with soils conditioned by more distant relatives as opposed to their own (i.e., negative plant-soil feedback), while negative slopes indicate that species perform better when inoculated with soils conditioned by close relatives (i.e., positive plant-soil feedback). Correlations tested for significant relationships between survival and total biomass feedbacks and the species-level proportion data.

Results

We found no support for our first hypothesis that soils conditioned by species of each subgenus would differ in microbial abundance and richness. Mixed linear models detected no significant variation between subgenera in bacterial or fungal OTU richness or abundance, NO_3^- and NH_4^+ concentrations of conditioned soils, or aboveground biomass (Table 1). However, soil NH_4^+ did significantly vary among eucalypt species within subgenera, as indicated by the significant random term species within subgenus. Further, variation in soil NH_4^+ significantly influenced both bacterial and fungal community composition at the OTU and family levels (Table 2). Soil NO_3^- significantly influenced bacterial and fungal community composition at the family and OTU levels, respectively. In contrast, aboveground biomass did not significantly influence bacterial or fungal community composition at either taxonomic level.

Table 1. Results of mixed linear models examining for variation in aboveground biomass, NH_4^+, NO_3^- as well as the abundance and richness of fungal and bacterial operational taxonomical units (OTU) between eucalypt subgenera. Bold values indicate statistical significance at $\alpha = 0.05$.

	Subgenus		Species within Subgenus*	
Factor	$\mathbf{F_{1,12}}$	***P***	$\boldsymbol{\chi^2}$	***P***
Aboveground biomass	2.3	0.154	2.0	0.155
NH_4^+	0.6	0.446	5.6	**0.018**
NO_3^-	0.5	0.481	2.1	0.151
Fungal OTU abundance	0.9	0.371	0.0	0.851
Fungal OTU richness	0.0	0.980	2.3	0.133
Bacterial OTU abundance	4.7	0.052	0.0	0.999
Bacterial OTU richness	0.0	0.969	0.0	0.999

*Significance determined by using log-likelihood tests comparing models with and without the random term species within subgenus.

Table 2. Results of distance based linear models (DistLM) examining the influence of NH_4^+, NO_3^- and aboveground biomass on fungal and bacterial community composition at both the operational taxonomic unit (OUT) and family levels. The model was applied to the Bray-Curtis matrix of among-pot similarities and the percentage of variation explained (%) by each variable is also shown. Bold values indicate statistical significance at $\alpha = 0.05$.

	NH_4^+			**NO_3^-**			**Aboveground biomass**		
Community	***Pseudo-F_{26}***	***P***	***%***	***Pseudo-F_{26}***	***P***	***%***	***Pseudo-F_{26}***	***P***	***%***
Fungal OTUs	2.3	**0.001**	8.0	1.4	**0.011**	5.2	1.3	0.116	4.6
Fungal family	2.5	**0.002**	8.8	1.4	0.091	5.0	1.3	0.202	4.6
Bacterial OTUs	1.9	**0.017**	6.6	1.2	0.117	4.5	1.2	0.141	4.6
Bacterial family	1.9	**0.021**	7.6	1.8	**0.043**	6.4	1.2	0.252	4.4

In support of our second hypothesis, the microbial community composition of conditioned soils differed between eucalypt subgenera (Table 3). After taking into account the significant effects of NH_4^+ on bacterial and fungal community composition, subgenus significantly influenced fungal community composition at both the OTU and family levels (PERMANOVA; p = <0.05), but not bacterial community composition. In the OTU model, NH_4^+ explained 4.8% of variation in fungal community composition, while subgenus explained 5.2%. In the family model, NH_4^+ explained 5.8% of variation in the composition of fungal families, while subgenus explained 6.2%. Further, canonical analysis of principal coordinates (CAP) successfully separated conditioned fungal communities by subgenus at each taxonomic level, particularly the OTU taxonomic level (figure 1). These factors tended to represent independent directions of variation. For instance, soil NH_4^+ explained CAP 1, with a maximum linear correlation coefficient of -0.99 for both fungal OTUs and families, while subgenus explained CAP 2, with a maximum linear correlation coefficient of 0.99 for both fungal OTUs and families.

Table 3. Results of permutational multivariate analysis of variance (PERMANOVA) models examining for variation in fungal and bacterial communities between eucalypt subgenera at both the operational taxonomic unit (OUT) and family levels. Community similarity among pots was assessed using a matrix of Bray-Curtis similarities based on presence absence data. Ammonium is fitted as a covariate in each model, subgenus as a fixed effect and species within subgenus as random. The proportion of variation explained by each component of the model was calculated from the variance components estimated by each model. Bold values indicate statistical significance at $\alpha = 0.05$.

Community	NH_4^+			Subgenus			Species within Subgenus		
	Pseudo- $F_{1,17}$	***P***	***%***	***Pseudo-*** $F_{1,12}$	***P***	***%***	***Pseudo-*** $F_{12,13}$	***P***	***%***
Fungal OTUs	2.3	**0.007**	4.8	1.5	**0.040**	5.2	1.0	0.417	1.4
Fungal family	2.5	**0.005**	5.8	1.7	**0.034**	6.2	1.1	0.337	3.2
Bacterial OTUs	1.8	**0.049**	3.1	1.2	0.161	1.8	1.0	0.385	2.0
Bacterial families	2.0	**0.034**	4.2	1.0	0.399	<0.1	1.2	0.089	13

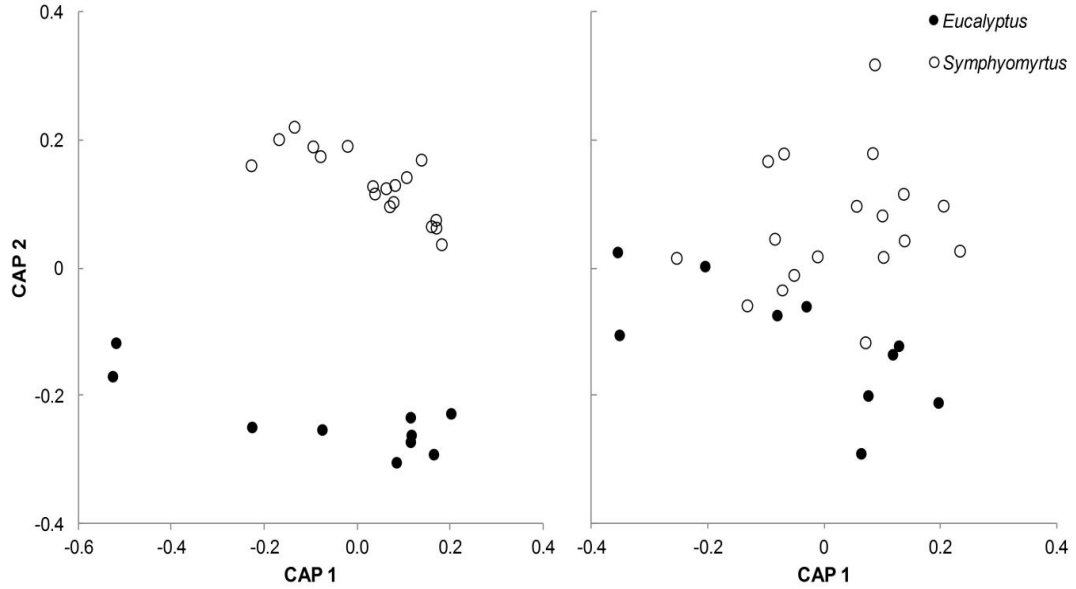

Figure 1. Canonical analyses of principal coordinates (CAP) plot of the effects of subgenus and NH_4^+ on fungal operational taxonomical units (OTUs; left) and families (right). The pot-level data is shown, where CAP1 is mainly aligned with variation in soil NH_4^+ concentration, whereas CAP 2 is mainly separated by the two subgenera.

Analysis of similarities (SIMPER) and generalised mixed linear models identified several key fungal families that significantly varied in their presence within soils conditioned by each subgenus (Table 4). The first ten fungal families identified by SIMPER contributed 10% to dissimilarity in fungal community composition between the subgenera. Further, generalised mixed linear models detected significant differences in the presence/absence of four families in soils conditioned by each subgenus. These families were: Fistulinaceae, unidentified family(s) belonging to the order Hysterangiales, and Davidiellaceae. The first of these families was present in a significantly greater number of subgenus *Eucalyptus* soils, while the latter two were present in a significantly greater number of subgenus *Symphyomyrtus* soils. The fourth was an unidentified family(s) from order Microascales, and was present in most subgenus *Eucalyptus* soils, but completely absent in the soils of subgenus *Symphyomyrtus*, and hence, could not be analysed with a generalised mixed linear model.

Table 4. Results of an analysis of similarities (SIMPER) identifying fungal families that explain the greatest amount of dissimilarity between the subgenera and the results of generalised mixed linear models analysing for variation in the presence/absence of each taxa in soils conditioned by each subgenus using pot-level data. The families explaining the first 10% of dissimilarity between subgenera are reported. Bold values indicate statistical significance at $\alpha = 0.05$.

Family	**Contribution (%)**	**Presence in subgenus *Eucalyptus* soils (%)**	**Presence in subgenus *Symphyomyrtus* soils (%)**	**Significance**	
				χ^2_1	P
Fistulinaceae	1.2	70	11	7.3	**0.007**
Order Hysterangiales family unclassified	1.1	20	67	5.1	**0.023**
Order Microascales family unclassified	1.1	60	0	-	-
Davidiellaceae	1.1	30	72	3.9	**0.047**
Kappamycetaceae	1.0	70	33	3.1	0.080
Bondarzewiaceae	1.0	60	22	2.7	0.102
Onygenaceae	1.0	40	72	2.4	0.123
Leotiomycetes family *Incertae sedis*	1.0	70	39	1.4	0.241
Marasmiaceae	1.0	60	33	1.5	0.224
Hymenochaetaceae	1.0	70	44	1.5	0.221

- We detected no unclassified order Microascales family(s) in the soils of subgenus Symphyomyrtus species, and thus, these taxa could not be analysed with a generalised mixed linear model.

In support of our third hypothesis, linear models detected significant relationships between the presence of key fungal taxa in the soils of eucalypt species and plant-soil feedbacks (Table 5). Subgenus had a large and significant effect on survival and biomass feedbacks, both with tau values of 0.72. Fistulinaceae and unclassified Microascales were both significantly correlated with survival feedbacks, with tau values of 0.57 and 0.5, respectively. The relationships were positive, indicating that the presence of these families in conditioned soils was associated with feedbacks becoming negative. All four of the families that differed in their presence/absence between the soils of each subgenus were significantly associated with biomass

feedbacks. Fistulinaceae and unclasssified Microascales both displayed positive relationships with biomass feedbacks, with tau values of 0.57 and 0.54, respectively. In contrast, unclassified Hysterangiales and Davidiellaceae displayed negative relationships, with tau values of -0.60 and -0.47, indicating that the presence of these taxa was associated with feedbacks becoming positive.

Table 5. Results of two-tailed Kendall rank correlations (tau) analysing for relationships between the proportion of pots of each species in which each fungal family was detected and the slopes (β) of linear relationships between the survival and total biomass of each species growing in soils inoculated with each conditioned soil compared to its own and phylogenetic distance between seedling and conditioning species. Subgenus differences are also shown, where a positive tau indicates that slopes are higher in subgenus *Eucalyptus*. Bold values indicate statistical significance at $\alpha = 0.05$.

	Seedling survival (β)			**Seedling biomass (β)**		
Predictor	**tau**	**Direction**	**P**	**tau**	**Direction**	**P**
Fistulinaceae	0.57	+	**0.019**	0.57	+	**0.019**
Order Hysterangiales family unclassified	0.40	-	0.102	0.60	-	**0.013**
Order Microascales family unclassified	0.50	+	**0.041**	0.54	+	**0.028**
Davidiellaceae	0.38	-	0.115	0.47	-	**0.049**
Subgenus	0.72	+	**0.004**	0.72	+	**0.004**

Discussion

We show that plant evolutionary history can influence soil fungal conditioning. Our data provide clear evidence that the eucalypt subgenera, *Eucalyptus* and *Symphyomyrtus*, conditioned statistically distinct soil fungal communities. While bacteria did not respond to eucalypt subgenus, fungal community composition did and this response occurred at both the OTU and family taxonomic levels. Several studies show that plant evolutionary history can explain variation in at least some fraction of soil microbial communities (i.e., bacterial and fungal communities or arbuscular or ectomycorrhizal fungi) under field conditions (Burns et al. 2015; Lugo et al. 2015; Tedersoo et al. 2013). However, an issue with such field-based studies is that phylogenetic effects may in part be confounded by environmental variation, as closely related species can occupy similar niches (Baldeck et al. 2013), possibly containing similar microbial communities. Also, there is evidence that spatial variation in soil microbial communities can influence the establishment of plant species (Reinhart et al. 2003). By growing same-age tree species in a uniform potting mix within a replicated and randomised common garden, we were able to demonstrate microbial conditioning without such confounding effects. Further, the observed conditioning effects manifested after a relatively short timeframe of just two years.

Phylogenetic signal in fungal community composition occurred through phylogenetically conserved mechanisms. Plant species often vary in growth strategy and nutrient use, both of which can influence soil microbial communities (Baxendale et al. 2014; Orwin et al. 2010). However, despite previous studies showing significant differences in the growth of subgenus *Eucalyptus* and *Symphyomyrtus* (Davidson and Reid 1980), at the age studied we detected no significant variation in aboveground biomass between the subgenera in the present study. Further, we detected no significant differences in the NH_4^+ or NO_3^+ content of soils conditioned by each subgenus, indicating no differences in N use. Thus, the mechanisms by which the subgenera differentially conditioned soil microbes within the present study are unknown. One potential mechanism that we did not investigate, however, was the influence of variable plant chemistry on soil microbes. Plant chemical compounds can influence soil microbial communities (Baetz and Martinoia 2014; Ehrenfeld et al. 2005) when released to soils via leaf litter or roots. In the present

case, there was very little leaf litter in the pots, but chemical compounds may have been introduced to soils via roots. While the foliar chemical composition of the subgenera is known to differ (Eschler et al. 2000; Li and Madden 1995; Wallis et al. 2010), only basic information is known regarding the root chemistry of eucalypts, besides the presence of phenolics (Jackson et al. 2000; Ling-Lee et al. 1977). Thus, whether the roots of the subgenera vary in chemical composition warrants attention.

We identified four ecologically important fungal taxa that contributed significantly to variable fungal community composition between the eucalypt subgenera. The most important family explaining dissimilarity in fungal community composition between the eucalypt subgenera was *Fistulinaceae*. Taxa belonging to this family were present in a significantly greater number of *Eucalyptus* as opposed to *Symphyomyrtus* conditioned soils and belonged exclusively to the genus *Fistulina*. This genus is known to cause rot of living heartwood in the butt and major roots of several *Eucalyptus* species, most of which belong to subgenus *Eucalyptus* (Keane et al. 2000). The second most important family was an unidentified Hysterangiales family(s), which was present in a significantly greater number of *Symphyomyrtus* as opposed to *Eucalyptus* conditioned soils. These taxa are commonly referred to as false-truffles (Hosaka et al. 2006) and form obligate ectomycorrhizal relationships with a wide range of trees, including eucalypts (Claridge 2002). The third most important family was an unclassified Microascales family(s), which occurred in samples of most subgenus *Eucalyptus* soils, but was completely absent in samples of soils conditioned by subgenus *Symphyomyrtus*. The Microascales are an order of mostly saprobic fungi that live in soil, where they break down vegetation. However, the order also contains some important fungal pathogens of trees, including eucalypts. For instance, species belonging to the genus *Ceratocystis* have been reported to cause wilt and canker on eucalypt species (Kamgan Nkuekam et al. 2013). Lastly, the family Davidiellaceae was present in a significantly greater number of *Symphyomyrtus* than *Eucalyptu*s soils. The Davidiellaceae taxa identified in this study belonged almost exclusively to a genus of moulds, *Cladosporium*, which can be pathogenic to plant foliage. For example, *Cladosporium herbarum* is a foliar pathogen of eucalypts and has been isolated from a number of species (Keane et al. 2000). However, no recorded cases of *Cladosporium spp.* attacking the belowground tissues of eucalypts were found. This variation in the presence of pathogenic and

mutualistic taxa between soils conditioned by each subgenus could potentially produce differential plant-soil feedbacks.

Phylogenetic signal in soil microbial conditioning may explain phylogenetic patterns in plant-soil feedbacks. Recent studies have shown a phylogenetic signal in plant-soil feedbacks, where closely related plant species display more similar feedback effects than expected to occur randomly (Anacker et al. 2014). For instance, Anacker et al. (2014) found experimental evidence of a significant phylogenetic signal in whole-soil feedbacks (as opposed to inoculations) within a Canadian old field community containing 57 species. However, because seedlings in the test phase were grown in whole conditioned soils, whether feedbacks were driven by microbial or chemical conditioning (e.g., phylogenetic patterns in nutrient use) could not be determined. In a recent study, we found a phylogenetic signal in plant-soil feedbacks using the same eucalypt species as this study (Chapter 3). Specifically, species from subgenus *Eucalyptus* performed better when inoculated with soils conditioned by more distant relatives as opposed to conspecifics or close relatives (i.e., displayed a phylogenetic Janzen-Connell effect; Liu et al. 2012), while species from subgenus *Symphyomyrtus* either showed neutral or small negative responses to inoculation with soils conditioned by more distant relatives. As we used an inoculum-based method to exclude the influence of conditioning effects on soil chemical properties (Brinkman et al. 2010; Kulmatiski and Kardol 2008), soil microbes were implicated as the causal agent. In particular, the phylogenetic Janzen-Connell effect exhibited by subgenus *Eucalyptus* species suggested that the conditioning trees of this subgenus had accumulated host-specific soil pathogens. By showing subgeneric variation in fungal community conditioning and significant relationships between key ecologically important taxa and plant-soil feedbacks, the present study provides strong evidence that differential microbial conditioning contributed to a phylogenetic signal in eucalypt plant-soil feedbacks. In particular, a significantly greater number of subgenus *Eucalyptus* as opposed to *Symphyomyrtus* conditioned soils were inhabited by *Fistulina* species that were significantly and positively correlated with plant-soil feedbacks becoming negative. On the other hand, the ectomycorrhizal fungi belonging to the order Hysterangiales were significantly and negatively associated with feedbacks, indicating that they may have contributed to the insignificant but small positive feedbacks observed in *Symphyomyrtus* species.

Conclusions

We provide clear evidence of a phylogenetic signal in fungal community conditioning. However, further research is required to investigate the potential drivers of the observed phylogenetic signal, in particular root chemistry. Our results show that microbial conditioning is likely an underlying mechanism of phylogenetic patterns in plant-soil feedback. We found that soils sampled from subgenus *Eucalyptus* more frequently contained the fungal pathogen *Fistulina sp.*, which was significantly correlated with phylogenetic Janzen-Connell effects observed in subgenus *Eucalyptus* species. However, future research should inoculate seedlings from each subgenus with *Fistulian sp.* to confirm the causality in this relationship. The findings of this study argue that soil microbial conditioning is dependent on plant evolutionary history, with potential performance consequences for themselves and co-occurring species.

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Chapter 5
Evolutionary history explains variation in the root chemistry of *Eucalyptus* species

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John K. Senior, Brad M. Potts, Noel W. Davies, Rachel C. Wooliver, Jennifer A. Schweitzer, Joseph K. Bailey, Julianne M. O'Reilly-Wapstra. Evolutionary history explains variation in the root chemistry of *Eucalyptus* species.

Summary

Plants are dependent on their root systems for survival and thus are defended from belowground enemies by a range of strategies, including plant secondary metabolites. These compounds vary among species and an understanding of this variation may provide generality in predicting the susceptibility of forest trees to belowground enemies and the quality of their organic matter inputs to soil. Here, we investigated phylogenetic patterns in the root chemistry of species within the genus *Eucalyptus*. Given the known diversity of plant secondary metabolites in eucalypt foliage, we hypothesized that (i) the range and concentrations of plant secondary metabolites and carbohydrates in roots vary among *Eucalyptus* species and (ii) that phylogenetic relationships explain a significant component of this variation.

To test for interspecific variation in root chemistry and the influence of tree evolutionary history, we grew 24 *Eucalyptus* species representing two subgenera (*Eucalyptus* and *Symphyomyrtus*) in a common garden for two years. Fine root samples were collected from each species and analysed for total phenolics, condensed tannins, carbohydrates, terpenes and formylated phloroglucinol compounds. Compounds displaying significant interspecific variation were mapped onto a molecular phylogeny and tested for phylogenetic signal.

Although all targeted groups of compounds were present, we found that phenolics dominated root defences and all phenolic traits displayed significant interspecific variation. Further, these compounds displayed significant phylogenetic signal. Overall, our results suggest that within these representatives of genus *Eucalyptus*, more closely related species have more similar root chemistry, which may influence their susceptibility to belowground enemies and soil organic matter accrual.

Introduction

Shared evolutionary history is emerging as an important predictor of plant ecological interactions. Plant traits commonly display phylogenetic signal, where close relatives share more similar traits than expected at random (Kraft and Ackerly 2010; Yang et al. 2014). Traits implicated in plant ecological interactions may also show such phylogenetic signals (Agrawal et al. 2009b; Johnson et al. 2014; Pearse and Hipp 2009). Thus, it is not unexpected that related species which share evolutionary conserved traits may also share similar interactions with their abiotic and biotic environment. Indeed, the evolutionary relationships among plant species can predict a range of ecological interactions, including those belowground. For example, phylogenetic signal has been detected in susceptibility to belowground herbivores and pathogens (Liu et al. 2012; Tippett et al. 1985; Vannette and Rasmann 2012), plant-mycorrhizal associations (Anacker et al. 2014; Reinhart et al. 2012; Tedersoo et al. 2013) and plant-soil feedback (Anacker et al. 2014). Although phylogenetic signal in plant chemistry has been implicated as an underlying driver of plant susceptibility to aboveground herbivores and pathogens (Carrillo-Gavilán et al. 2015; Johnson et al. 2014; Pearse and Hipp 2009), whether this extends to belowground plant-antagonist interactions remains unclear.

Reviews confirm that, like aboveground tissues, roots are also defended by a range of plant secondary metabolites including alkaloids, glucosinolates, phenolics, terpenoids, furanocoumarins and cardenolides (Rasmann and Agrawal 2008; van Dam 2009). Many of these compounds have been linked directly to plant defence against soil enemies (Baetz and Martinoia 2014; van Dam 2009), most notably, phenolics and terpenoids (Lanoue et al. 2010; Wurst et al. 2010). For example, phenylpropanoids are a group of plant phenolics that occur in defensive exudates secreted from the roots of barley as a rapid defence response to infection with the fungal pathogen *Fusarium graminearum* (Lanoue et al. 2010). Root compounds may also have important 'afterlife' effects when roots decompose, influencing the quantity and quality of soil organic matter (Freschet et al. 2013). For example, higher condensed tannin concentrations, in foliage at least, have been associated with slower rates of decomposition (Coq et al. 2010; Wardle et al. 2002), due to their ability to bind to proteins or cell wall components forming less degradable complexes (Cai et al. 1989; Mutabaruka et al. 2007; Northup et al. 1995). Once

released into soils via decomposition, these compounds may then affect soil nitrogen (N) mineralisation. For example, Schweitzer et al. (2004) found that genotypic variation in the condensed tannin inputs of cottonwood foliage could explain 55-65% of variation in soil N mineralisation. Thus, quantifying variation in root chemical traits across species could contribute to our understanding of plant susceptibility to belowground enemies as well as ecosystem processes.

The genus *Eucalyptus* is an ideal system to assess the influence of plant evolutionary history on root chemical traits. *Eucalyptus* species dominate many of Australia's ecosystems and provide habitat or resources for a range of dependent organisms including foliar pathogens and aboveground invertebrate and mammal herbivores (e.g., Borzak et al. 2015; Matsuki et al. 2011; Wingfield et al. 2008). As such, eucalypt foliage is chemically defended by a range of plant secondary metabolites, most notably, terpenes, cyanogenic glycosides and phenolics (Gleadow et al. 2003; Mann et al. 2012; McKiernan et al. 2014; Moore and Foley 2005), including formylated phloroglucinol compounds (FPCs), a group of phenolic compounds with strong anti-herbivore properties (Matsuki et al. 2011; Moore and Foley 2005; O'Reilly-Wapstra et al. 2004). Eucalypts are also susceptible to a range of belowground organisms (Kile et al. 1979; Wilcken et al. 2002; Wingfield et al. 2008), including the fungal pathogen *Phytophthora cinnamomi*, to which species from subgenus *Eucalyptus* are more susceptible than species within subgenus *Symphyomyrtus* (Podger and Batini 1971; Tippett et al. 1985). One potential driver of this pattern may be divergence in root chemistry, yet only basic information is known regarding the root chemical defences of eucalypts, besides the presence of phenolics (Jackson et al. 2000; Ling-Lee et al. 1977). By utilising 24 of the 30 *Eucalyptus* species occurring in Tasmania, the island state of Australia, belonging to both subgenus *Eucalyptus* and subgenus *Symphyomyrtus*, we tested the hypotheses: (i) the presence and concentrations of plant secondary metabolites and carbohydrates would display significant variation among species and (ii) this variation in secondary compounds and carbohydrates would exhibit significant phylogenetic signal.

Materials and methods

Common pot experiment

We established a common pot experiment to analyse genetic variation in root chemical traits among eucalypts. Open-pollinated seed of 24 native Tasmanian eucalypt species was obtained from Forestry Tasmania (http://www.forestrytas.com.au/), wet stratified and germinated following Senior et al. (2013). Ten species were available from subgenus (subg.) *Eucalyptus* and 14 species from subg. *Symphyomyrtus*. Phylogenetic analysis (described below) further resolved phylogenetic relationships among these species, identifying five genetic lineages below the subgenus level (see table 2 and figure 1). Twelve seedlings of each species were transplanted from germination trays and grown in forestry tubes filled with commercial potting soil. Potting soil consisting of eight parts composted pine bark and three parts coarse river sand with added macro- and micro-nutrients from Nutricote Grey (Langley Australia Pty Ltd., Welshpool WA), which included nitrogen (N), phosphorus (P) and potassium (K) in the weight ratio of 19:2.6:10. After 6 months, six individuals of each species were planted, spaced evenly apart, in each of two 33 L replicate pots. After 2 additional years of growth in a completely randomised design, the trees were destructively harvested to collect root material. Trees were severed at the root collar and soil was shaken from their roots. From each pot, five randomly sampled balls of lateral and fine root material were then taken from the combined root mass of all six trees and pooled. Samples were immediately placed in a cooler with ice and later rinsed to remove soil before storage at -4°C for chemical analysis.

Chemical analyses

To determine whether eucalypt species differ in root chemical traits, we quantified total phenolics, non-structural carbohydrates, FPCs and terpenes using individuals grown in the common pot experiment. Studies show that root size class may influence root chemistry (van Dam 2009). Our root samples varied in diameter from 0.5-5 mm, however, the majority of sample collected from each species was within the fine root fraction (≤ 2 mm). Therefore, for consistency, all chemical assays were conducted on fine roots that were living when collected, as determined by their colour. For the analysis of total phenolics, non-structural carbohydrates and FPCs,

freeze-dried root samples were ground to a fine powder using a Cyclotec^{TM} 1093 cyclone mill (Foss, Hillerød, DK), whereas terpenes were extracted from thawed root material. For all chemical traits, the extraction of the root sample collected from each pot was duplicated, giving a total of 96 extracts (24 species x 2 pots x 2 extractions) per assay. A portion of each root sample was weighed, oven-dried for 48 hours at 60 °C and then re-weighed to calculate the proportion of dry mass in each sample, which was then used to convert all chemical concentrations to dry mass equivalents.

Phenolics were extracted from approximately 0.25 g of root sample and quantified in duplicate following a modified Prussian Blue assay (Graham 1992) that can be found in Ann Hagerman's web-based tannin handbook (http://www.users.miamioh.edu/hagermae/). The absorbance of extracts was read at 700 nm using a spectrophotometer (Vis 7200A, Techcomp, Shanghai, China) and the concentrations of total phenolics were quantified using gallic acid (0, 6.8, 13.6, 20.4, 27.2 and 34.0 μg ml^{-1}) as an external standard (Sigma, G-7384). Concentrations of total phenolics were expressed as mg g^{-1} DM gallic acid equivalents. The same phenolic extracts were also used to quantify condensed tannins in duplicate following a modified Acid Butanol assay (Porter et al. 1985), that can be found in Ann Hagerman's web-based tannin handbook. Absorbance was read at 550 nm on a UV-vis spectrophotometer (Hitachi U-1800, Tokyo, Japan) and the concentrations of condensed tannins were quantified using sorghum tannin (0, 0.4, 0.6, 0.8 and 1.0 mg ml^{-1}) as an external standard, as recommended. Sorghum tannins were extracted from grain and purified following methods outlined in Hagerman and Butler (1980). This method specifically targets sorghum tannins and is designed to reduce contaminant proteins to 2-3% of dry extract weight. Concentrations of condensed tannins were expressed as mg g^{-1} DM sorghum tannin equivalents.

Total non-structural carbohydrates were extracted from 50 mg of root sample. Soluble sugars and starch were quantified in duplicate following a modified phenol-sulphuric acid method outlined in Page et al. (2013). Absorbance was read at 490 nm and the concentrations of soluble sugars and starch were quantified using glucose (0, 10, 20, 40, 60, 80 and 100 μg ml^{-1}) as an external standard (Sigma-Aldrich, G8270-100G). Concentrations of soluble sugars and starch were expressed as mg g^{-1} DM glucose equivalents and were summed to yield total non-structural carbohydrates (Page et al. 2013). Hereafter, soluble sugars are referred to as sugars.

Terpenes were extracted from 1 g of thawed root material, sliced transversely into 1-2 mm segments, using methods modified from O'Reilly-Wapstra et al. (2004). Sliced samples were extracted using a stock solution of dichloromethane containing 100 µg L^{-1} of n-heptadecane as an internal standard. Extracts were then analysed by combined gas chromatography-mass spectrometry (GC-MS) on a Varian CP-3800 gas chromatograph with a split/splitless injector coupled to a Bruker 300-MS quadrupole mass spectrometer. The column was an Agilent DB5-MS (30m x 0.25mm x 0.25 microns), the carrier gas was helium at 1.5mLs/min, the injector temperature was 220 °C, and the oven temperature was programmed, after a 1 minute hold at 60 °C, to 240 °C at 12 °C per minute then to 280 °C at 30 °C per minute. One microlitre was injected with a 4:1 split ratio. Nineteen compounds were identified through the NIST and in-house databases and Kovats' Indices and quantified using Bruker MS Workstation software (see Appendix B, Figure B1). Seventeen of these compounds were terpenes, while two were acylphloroglucinol compounds that could not be further identified. These compounds are hereafter referred to as unknown 1 and 2. Results were expressed as mg g^{-1} DM for 1,8-cineole using a reference standard (Sigma-Aldrich, 00020590-100MG), while the other terpene components identified were expressed as mg g^{-1} DM cineole equivalents. The concentrations of the individual terpene compounds detected within samples were summed to provide a measure of the total quantifiable terpenes, hereafter referred to as total terpenes.

Formylated phloroglucinol compounds were extracted using a stock solution of acetonitrile and quantified by high performance liquid chromatography (HPLC), as outlined in Wallis and Foley (2005). We measured three specific FPCs, sideroxylonal A, sideroxylonal C and macrocarpal A, and a group of later eluting compounds that could not be clearly resolved, which are hereafter referred to as other FPCs. This later group mainly comprised sesquiterpene containing macrocarpals, as determined by UV-Vis spectroscopy and mass spectra, with a single major unidentified sesquiterpene macrocarpal eluting well before macrocarpal G, dominating this group in most cases. This compound was able to be individually quantified and is hereafter referred to as unknown FPC. Sideroxylonal A and C and macrocarpal A were identified using reference standards (see Eyles et al. (2003) for details) and are expressed as mg g^{-1} DM, while other FPCs and the unknown FPC

were expressed as mg g^{-1} DM equivalents of macrocarpal A. For each sample, the targeted FPCs (sideroxylonal A, C, and macrocarpal A, other FPCs and the unknown FPC) were summed to provide a measure of total targeted FPCs, hereafter referred to as total targeted FPCs.

Phylogenetic analysis

We constructed a phylogeny of the eucalypt species used in this experiment using Diversity Arrays Technology (DArT) markers, which have previously been used to resolve species-level phylogenetic relationships in eucalypts (Steane et al. 2011). We used presence/absence data from a set of 3,885 DArT markers which were polymorphic across three samples of 26 eucalypt species (25 are native to Tasmania and one, *E. nitens*, is closely related but not native to Tasmania). The Tasmanian species included samples from the same seedlots as used in the pot experiment. A consensus dataset was compiled by Diversity Arrays Technology Pty. Ltd., Yarralumla, ACT, Australia (http://www.diversityarrays.com). For each marker, species were given a 0 if all 3 genotypes of that species scored 0, a 1 if all 3 genotypes of that species scored a 1, or treated as missing data if polymorphic. Given that the data were binary, they were analysed as discrete character data (Lewis 2001). Marker data were analysed using Bayesian Evolutionary Analysis by Sampling Trees (BEAST version1.75; (Drummond and Rambaut 2007). We ran 4 chains of 100,000,000 steps with sampling every 1000th step and estimating the rate of evolution from a normal distribution around 1 using BEAUti version 1.7.5. The root of the consensus phylogeny was assigned an arbitrary age of one. All 4 chains reached convergence on the same posterior distribution and were combined into a single tree file using LogCombiner version 1.7.5. From these trees we developed a consensus phylogeny by removing the first 100,000 (25%) sampled trees as burn-in and calculating posterior probabilities at each node using TreeAnnotator version 1.7.5. The tips of this phylogeny were subsequently pruned to match the subset of species for which root chemistry data were available for the analyses described below.

Statistical analysis

For all chemical traits, we averaged the two extractions per pot to remove pseudoreplication. We fitted linear models in R (R Development Core Team 2014)

to test for interspecific variation in the concentrations of total phenolics, condensed tannins, sugars, starch, total non-structural carbohydrates, total terpenes and FPCs using the function lm within the package *stats* (R Development Core Team 2014). The anova function from the package *stats* was used to conduct F tests on each model.

To determine whether significant interspecific variation in root chemical traits was influenced by eucalypt evolutioaary history, we mapped the estimated species means for each trait onto the eucalypt phylogeny and calculated Blomberg's K (Blomberg et al. 2003) using the function phylosig within the package *phytools* (Revell 2012). Blomberg's *K* is a measure of phylogenetic signal, where K values that are significantly greater than 0 indicate that closely related species resemble each other more than they resemble species drawn at random from a given phylogenetic tree. We calculated phylogenetic signal of chemical traits for the whole phylogeny, then within each subgenus, to determine whether phylogenetic structuring of these traits varies across phylogenetic scales. In our calculations of K, we incorporated variation between replicate pots (i.e. standard errors of species least squares means) which accounted for measurement error, which includes within-species variation, plasticity and instrument-related error (Ives et al. 2007). We lacked DArT data for one species, *Eucalyptus delegatensis*, thus it was not included in phylogenetic analyses. Phylogenetic signal in root chemical traits was presented by mapping species deviations from the overall mean of traits onto the eucalypt phylogeny using the function plot.phylo within the package *ape* (Paradis et al. 2012).

To understand the drivers of phylogenetic signals, we fitted mixed linear models testing for variation in chemical traits between subgenera and among lineages using the function lme within the package *lme4* (Bates et al. 2015). We used the random terms 'species within subgenus' or 'species within lineage' to test subgenus and lineage fixed effects, respectively. *Eucalyptus globulus* and *E. gunnii* were removed from the lineage-level analyses, as our placement of these species was inconsistent with previous phylogenetic analyses (McKinnon et al. 2008; Steane et al. 2011). The anova function from the package *lmerTest* (Kuznetsova et al. 2015) was used to conduct F tests on each model.

For all linear models, residuals were checked for homoscedasticity and normality using the function plot.lm from the package *stats* or the function anova from the

package *lmerTest* (Kuznetsova et al. 2015) for linear and mixed linear models, respectively. The diagnostic plots of each chemical trait were similar across species, lineage and subgenus-level models, and subsequently, the same transformations were used for each level of analysis. Total oils and total targeted FPCs required a log and cube root transformation, respectively. Starch was BoxCox transformed (λ = -0.8) using the function boxcox from the package *MASS* (Ripley et al. 2015). No other traits required transformation. The function predictmeans from the package *predictmeans* was used to conduct Tukey's HSD tests as well as acquire group means (back-transformed if necessary) for all linear models.

We tested for variation in individual terpenes and FPCs among species, lineages and subgenera. Individual compounds did not meet the assumptions of linear models due to zero inflation. Thus, in place of linear models described above, Kruskal-Wallis Rank Sums tests were used to test for variation among species, lineages and subgenera using the function kruskal.test within the package *stats*. In this case, pot averages (as used in linear models) were used to test for species differences, while species averages were used to test for lineage or subgenus differences.

Results

As predicted by Hypothesis 1, linear models confirm that root chemical traits vary considerably among species (Tables 1 and 2). Specifically, the concentrations of total phenolics, condensed tannins, sugars, total non-structural carbohydrates, total terpenes and total targeted FPCs significantly differed among species. Total phenolics ranged two-fold, from 51.7 mg g^{-1} DM in *E. johnstonii* to 97.7 mg g^{-1} DM in *E. radiata*. Condensed tannins ranged three-fold, from 49.3 mg g^{-1} DM in *E. tenuiramis* to153.3 mg g^{-1} DM in *E. brookeriana*. Sugars ranged two-fold, from 8.6 mg g^{-1} DM in *E. regnans* to 17.9 mg g^{-1} DM in *E. brookeriana*. Total non-structural carbohydrates ranged two-fold, from 11.4 mg g^{-1} DM in *E. regnans* to 29.2 mg g^{-1} DM in *E. brookeriana*. Significance in total non-structural carbohydrates was driven by significant variation in sugars, as starch concentrations did not significantly differ among species. We detected terpenes in the roots of all species, generally in very low concentrations, but ranging in two orders of magnitude from <0.01 mg g^{-1} DM in *E. pauciflora* to 1.68 mg g^{-1} DM in *E. cordata*; however, Tukey's pair-wise tests did not detect significant differences in terpenes. Eight species contained <0.01 mg g^{-1}

DM of FPCs within their roots, while five species had relatively high concentrations, ranging from 5.22 mg g^{-1} DM in *E. barberi* to 15.76 mg g^{-1} DM in *E. rubida*.

Table 1. Results of linear models examining interspecific variation in root chemical traits among 24 eucalypt species. Bold values indicate statistical significance (α = 0.05).

	Species	
Variable	**$F_{(23,24)}$**	**P**
Total phenolics	4.9	**<0.001**
Condensed tannins	4.5	**<0.001**
Sugars	3.2	**0.003**
Starch	1.7	0.107
Total non-structural carbohydrates	2.1	**0.040**
Total terpenes	3.4	**0.002**
Total targeted FPCs	56.9	**<0.001**

Table 2. Least squares means (back transformed if necessary) of root chemical traits for 24 eucalypt species as predicted by mixed linear models.

			Total phenolics	Condensed tannins	Sugars	Starch	Total non-structural carbohydrates	Total terpenes	Total targeted FPCs
Subgenus	**Lineage**	**Species**	**Mean mg g^{-1} DM**	**Mean mg g^{-1} DM**	**Mean mg g^{-1} DM**	**Mean mg g^{-1} DM**	**Mean mg g^{-1} DM**	**Mean mg g^{-1} DM**	**Mean mg g^{-1} DM**
Eucalyptus	**1**	*E. obliqua*	88.6^{abcd}	124.5^{abcde}	12.5^{ab}	4.8	17.7^{ab}	0.01	0.00^{d}
	1	*E. regnans*	69.9^{abcde}	70.3^{cde}	8.6^{b}	3.0	11.4^{b}	0.09	<0.01^{d}
	1	*E. sieberi*	95.4^{ab}	80.3^{abcde}	13.2^{ab}	4.7	17.6^{ab}	0.01	0.00^{d}
	1	*E. delegatensis*	86.9^{abcd}	119.2^{abcde}	14.4^{ab}	3.6	18.1^{ab}	0.01	<0.01^{d}
	1	*E. pauciflora*	73.9^{abcde}	81.6^{abcde}	9.6^{ab}	3.4	14.6^{ab}	<0.01	<0.01^{d}
	2	*E. tenuiramis*	76.5^{abcde}	49.3^{e}	15.1^{ab}	3.2	18.4^{ab}	0.01	0.01^{d}
	2	*E. risdonii*	85.7^{abcd}	54.9^{de}	15.6^{ab}	4.0	19.6^{ab}	0.01	0.00^{d}
	2	*E. nitida*	87.7^{abcd}	87.2^{abcde}	11.5^{ab}	4.4	15.9^{ab}	0.01	<0.01^{d}
	2	*E. radiata*	97.9^{a}	91.7^{abcde}	11.8^{ab}	2.3	14.1^{ab}	0.11	<0.01^{d}
	2	*E. pulchella*	78.2^{abcde}	77.1^{abcde}	10.1^{ab}	2.7	12.8^{ab}	0.03	0.00^{d}
	2	*E. amygdalina*	90.2^{abcd}	120.0^{abcde}	13.6^{ab}	5.7	19.4^{ab}	0.01	0.01^{d}
Symphyomyrtus	**NA**	*E. gunnii*	69.8^{abcde}	118.4^{abcde}	15.8^{ab}	5.7	21.5^{ab}	0.01	<0.01^{d}
	3	*E. brookeriana*	93.1^{abcd}	152.7^{ab}	17.9^{a}	10.2	29.2^{a}	0.04	0.92^{c}
	3	*E. rodwayi*	94.0^{abc}	153.3^{a}	14.9^{ab}	6.4	21.3^{ab}	0.19	0.16^{cd}
	3	*E. barberi*	84.2^{abcd}	121.0^{abcde}	10.8^{ab}	3.9	15.1^{ab}	0.30	5.22^{b}
	4	*E. urnigera*	65.6^{abcde}	108.0^{abcde}	12.0^{ab}	2.4	14.5^{ab}	0.02	<0.01^{d}
	4	*E. cordata*	60.9^{de}	68.5^{cde}	11.7^{ab}	3.9	15.7^{ab}	1.68	0.00^{d}
	4	*E. subcrenulata*	63.0^{bcde}	93.4^{abcde}	10.8^{ab}	2.7	13.4^{ab}	0.92	0.02^{d}
	4	*E. johnstonii*	51.7^{e}	74.7^{bcde}	9.4^{ab}	2.9	12.0^{ab}	0.01	<0.01^{d}
	NA	*E. globulus*	62.5^{cde}	94.8^{abcde}	9.6^{ab}	4.7	15.0^{ab}	0.49	0.00^{d}
	5	*E. perriniana*	71.2^{abcde}	102.2^{abcde}	14.8^{ab}	4.3	19.0^{ab}	0.54	5.59^{b}
	5	*E. dalrympleana*	76.4^{abcde}	140.3^{abc}	14.5^{ab}	6.8	22.6^{ab}	0.02	8.01^{ab}
	5	*E. viminalis*	73.3^{abcde}	131.1^{abcd}	16.9^{ab}	5.5	23.9^{ab}	0.34	10.58^{ab}
	5	*E. rubida*	72.1^{abcde}	106.7^{abcde}	17.1^{ab}	5.4	22.5^{ab}	0.08	15.76^{a}

Letters indicate significance (α=0.05) after Tukey's HSD pair-wise comparisons of variation among species for each trait and no lettering indicates insignificance after Tukey's adjustment. Total phenolics are expressed as gallic acid equivalents, condensed tannins are expressed as Sorghum tannin equivalents and total terpenes are expressed as 1,8-cineole equivalents.

As predicted by Hypothesis 2, variation in the concentrations of total phenolics, condensed tannins, total terpenes and total targeted FPCs displayed significant (P <0.05) phylogenetic signal across the phylogeny, with K ranging from 0.40 to 0.67 (Table 3 and Figure 1). When only *Symphyomyrtus* species were tested, total phenolics, condensed tannins, sugars and total targeted FPCs displayed significant phylogenetic signal, with K values ranging from 0.94 to 1.41 for significant compounds. Within subgenus *Eucalyptus*, no chemical trait displayed phylogenetic signal.

Table 3. Phylogenetic signal (Blomberg's K) of root chemical traits across 23 eucalypt species. Bold values indicate statistical significance ($\alpha = 0.05$)

	Whole phylogeny		**Subgenus *Symphyomyrtus***		**Subgenus *Eucalyptus***	
Trait	**P**	**K**	**P**	**K**	**P**	**K**
Total phenolics	**0.002**	0.44	**0.004**	1.24	0.696	0.69
Condensed tannins	**0.006**	0.40	**0.040**	0.95	0.435	0.74
Soluble sugars	0.055	0.38	**0.047**	0.94	0.257	0.95
Total terpenes	**0.023**	0.42	0.919	0.46	0.859	1.00
Total targeted FPCs	**0.001**	0.67	**0.002**	1.41	0.938	1.00

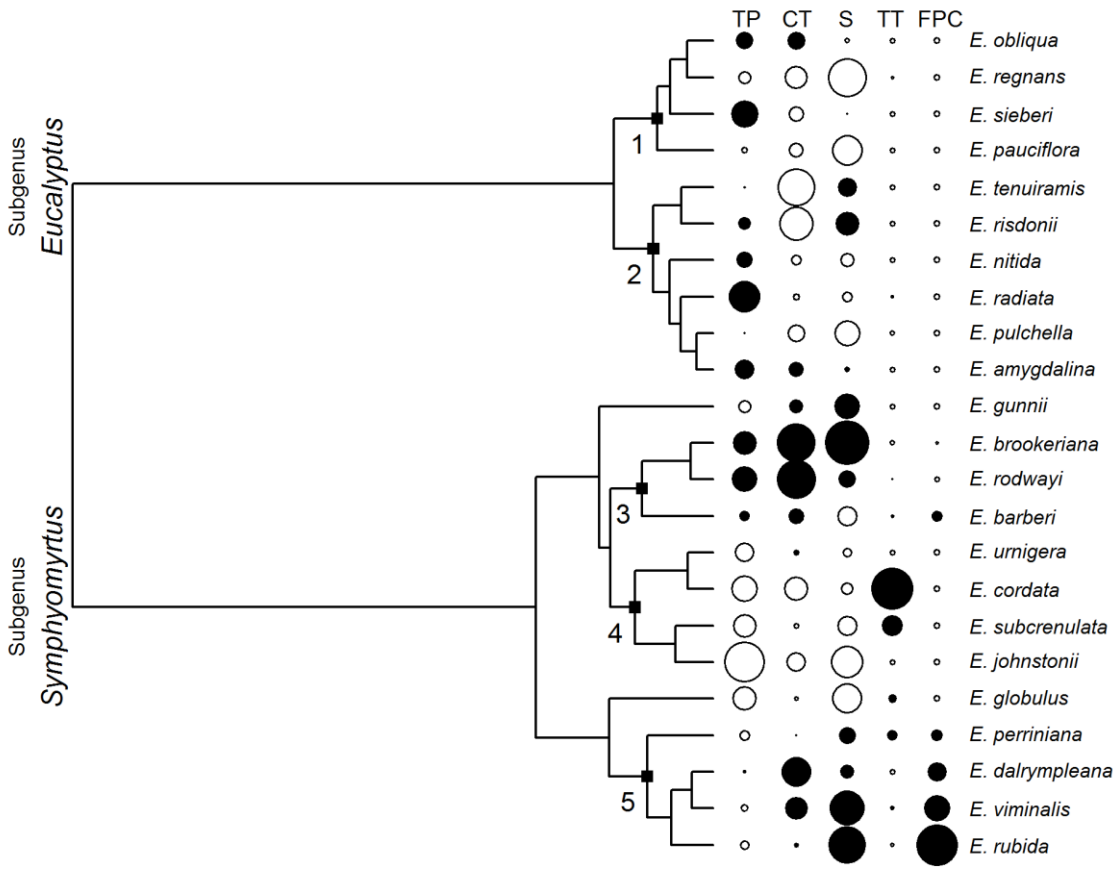

Figure 1. Phylogeny of the eucalypt species (subgenus *Eucalyptus* and *Symphyomyrtus*) used in the present study with mapped species deviations from the overall mean of total phenolics (TP), condensed tannins (CT), sugars (S), total terpenes (TT) and FPCs (FPC) calculated from least squares means. Full circles represent values above the overall mean, and open circles represent values below the overall mean. The size of circles represents the extent of deviation from the mean, where larger circles deviate more from the mean. Only traits showing significant phylogenetic signal are mapped. Phylogenetic lineages are labelled at the node of each respective lineage

Mixed linear models provide further support that root chemistry is phylogenetically structured (Tables 4 and 5). Overall, subgenus *Symphyomyrtus* species displayed significantly higher concentrations of condensed tannins, total terpenes and FPCs than subgenus *Eucalyptus*, while subgenus *Eucalyptus* species displayed greater concentrations of total phenolics (Table 4). Concentrations of condensed tannins, total terpenes and targeted FPCs were on average 1.3, 11 and 900 times greater in

subgenus *Symphyomyrtus*, than respective concentrations in subgenus *Eucalyptus* species, while total phenolics were on average 1.2 times greater in subgenus *Eucalyptus*. In accordance with subgenus-level tests of phylogenetic signal, phylogenetic lineages within subgenus *Eucalyptus* (lineages 1 and 2) did not differ in the concentrations of any chemical trait but lineages within *Symphyomyrtus* (lineages 3-5) did (Table 5). On average, species within lineage 3 exhibited 50% higher total phenol concentrations and 65% higher condensed tannin concentrations than those within lineage 4. Species from lineage 5 on average displayed 45% greater soluble sugar concentrations compared to species from lineage 4. Species belonging to lineage 5 also had 6.5- and 4000-fold higher concentrations of FPCs than those within lineages 4 and 3, respectively. *Symphyomyrtus* lineages did not differ in total terpene concentrations.

Table 4. Results of mixed linear models examining variation in root chemical traits between the eucalypt subgenera *Eucalyptus* and *Symphyomyrtus*, including the predicted least squares means for each subgenus (back transformed if necessary). Bold values indicate statistical significance ($\alpha = 0.05$).

	Total phenolics (mg g^{-1} DM)	**Condensed tannins (mg g^{-1} DM)**	**Soluble sugars (mg g^{-1} DM)**	**Total terpenes (mg g^{-1} DM)**	**Total targeted FPCs (mg g^{-1} DM)**
Eucalyptus	84.6	86.9	12.4	0.01	<0.01
Symphyomyrtus	72.1	112.7	13.6	0.11	0.85
$F_{(1,22)}$	7.7	5.7	1.2	11.2	8.6
P	**0.011**	**0.026**	0.292	**0.002**	**0.008**

Table 5. Results of mixed linear models analysing for variation in chemical traits among phylogenetic lineages, including the predicted least squares means for each lineage (back transformed if necessary). Bold values indicate statistical significance (α = 0.05).

	Total phenolics (mg g^{-1} DM)	**Condensed tannins (mg g^{-1} DM)**	**Soluble sugars (mg g^{-1} DM)**	**Total terpenes (mg g^{-1} DM)**	**Total targeted FPCs (mg g^{-1} DM)**
Lineage 1	82.9^{a}	95.2^{ab}	11.7^{ab}	<0.01	<0.01^{c}
Lineage 2	86.0^{a}	80.1^{b}	12.9^{ab}	<0.01	<0.01^{c}
Lineage 3	90.4^{a}	142.3^{a}	14.6^{ab}	0.02	1.27^{b}
Lineage 4	60.3^{b}	86.1^{b}	10.9^{b}	0.02	<0.01^{c}
Lineage 5	73.3^{ab}	120.1^{ab}	15.8^{a}	0.02	9.5^{a}
$F_{(4,17)}$	10.3	5.1	3.2	2.8	51.0
P	**<0.001**	**0.007**	**0.036**	0.058	**<0.001**

Letters indicate significance (P <0.05) after Tukey's HSD pair-wise comparisons of variation among species for each trait. Lineages are defined in Table 2.

Non-parametric ANOVA revealed significant variation among species, lineages, and subgenera in the concentrations of individual terpenes and FPCs (Table 6 and Appendix B, Table B1). The terpene compounds α-pinene, α-phellandrene, 1,8 cineole, γ-terpinene, aromadendrene and globulol were detected in one or more species at concentrations greater than 0.1 mg g^{-1} DM, but all other terpenes were detected in trace concentrations (< 0.1 mg g^{-1} DM). Alpha-pinene, α-phellandrene and 1,8 cineole tended to be the dominant terpene compounds occurring in eucalypt roots, occurring in at least 11 species, with species containing on average 0.03, 0.06 and 0.06 mg g^{-1} DM, respectively. The remaining compounds occurred in fewer than 11 (mostly *Symphyomyrtus*) species. Although only α-terpinene, ρ-cymene, α-terpineol and bicyclogermacrene significantly differed among species, and only 1,8 cineole, limonene and γ-terpinene differed among lineages, all compounds but globulol and unknown compound 1 differed between the subgenera. These compound concentrations were generally higher within subgenus *Symphyomyrtus* species. FPCs were almost exclusively detected in subgenus *Symphyomyrtus* species, but trace concentrations were detected in a few subgenus *Eucalyptus* species. The unknown FPC and the grouped other FPCs tended to dominate the targeted FPC compounds, where species containing these compounds had on average 1.6 and 4.86 mg g^{-1} DM, respectively. All FPC compounds significantly differed in concentration

among species, lineages and subgenera, with the exception of the unknown FPC which did not significantly differ between the subgenera.

Table 6. Results of non-parametric Kruskal-Wallis tests analysing for variation in individual terpenes, acylphloroglucinols and FPCs among species, lineages and subgenera. Bold values indicate statistical significance (α = 0.05).

	Species		**Lineage**		**Subgenus**	
Compound	$X^2_{(23)}$	***P***	X^2_4	***P***	$X^2_{(1)}$	***P***
Terpenes [a]						
α-pinene	34.5	0.058	7.0	0.138	6.9	**0.008**
α-phellandrene	32.9	0.082	8.1	0.090	7.9	**0.005**
α-terpinene	36.2	**0.039**	6.8	0.149	6.3	**0.012**
ρ-cymene	36.5	**0.037**	7.6	0.108	7.6	**0.006**
limonene	34.7	0.056	10.4	**0.034**	10.3	**0.001**
1,8-cineole	33.9	0.067	10.3	**0.036**	9.6	**0.002**
γ-terpinene	34.2	0.062	10.6	**0.032**	10.9	**<0.001**
terpinene-4-ol	34.7	0.056	6.2	0.186	5.5	**0.019**
α-terpineol	38.4	**0.023**	5.9	0.209	5.7	**0.017**
terpinyl acetate	34.3	0.061	8.6	0.072	6.6	**0.010**
α-gurjunene	34.5	0.058	9.1	0.058	9.3	**0.002**
aromadendrene	32.4	0.092	5.3	0.261	4.2	**0.040**
viridiflorene	32.7	0.085	4.1	0.398	4.5	**0.035**
bicyclogermacrene	38.9	**0.021**	6.3	0.180	7.0	**0.008**
globulol	35.0	0.052	4.9	0.293	3.3	0.067
viridiflorol	34.1	0.064	5.2	0.263	4.4	**0.036**
β-eudesmol	33.1	0.080	6.2	0.187	6.0	**0.014**
Acylphloroglucinols [b]						
unknown 1	31.7	0.106	2.4	0.668	1.8	0.175
unknown 2	33.8	0.068	4.7	0.316	4.8	**0.028**
FPCs [c]						
sideroxylonal A	39.3	**0.018**	15.2	**0.004**	6.1	**0.013**
sideroxylonal C	45.2	**0.004**	19.2	**<0.001**	9.2	**0.002**
macrocarpal A	40.6	**0.013**	16.2	**0.003**	6.6	**0.010**
unknown FPC	42.2	**0.009**	15.4	**0.004**	3.7	0.055
other FPCs	46.9	**0.002**	18.7	**<0.001**	6.3	**0.012**

[a] Identified by mass spectra and/or retention indices using gas chromatography-mass spectrometry (GC-MS).

[b] Identified by mass spectra using gas chromatography-mass spectrometry (GC-MS).

[c] Identified by reference standards, UV-Vis spectroscopy and mass spectra using high performance liquid chromatography (HPLC).

Discussion

Within the roots of eucalypt species, we found high concentrations of secondary metabolites implicated in plant defence and the quality of organic matter in soils. Moreover, we found significant phylogenetic structure in these compounds that varied between and within subgenera. Chemical defence in eucalypt roots is not well known, but studies have revealed the presence of phenolics in several species (Jackson et al. 2000; Ling-Lee et al. 1977). Two key findings emerged from this study: firstly, phenolics (total, condensed tannins and FPCs) dominated eucalypt root chemistry and secondly, phenolic compounds exhibited significant phylogenetic signal across the eucalypt subgenera and within subgenus *Symphyomyrtus*. This is important as it suggests that ecologically important root chemical traits may significantly vary among phylogenetic lineages, with possible implications for predicting the susceptibility of forest trees to belowground enemies as well as the quality of organic matter inputs to soil**.**

Our findings indicate that concentrations of phenolics within eucalypt roots may equal or even surpass those found in foliage. The concentrations of terpenes, phenolics and FPCs in eucalypt foliage have been extensively studied in our lab using the same standards and extraction and quantification procedures as the present study, allowing for basic comparisons to previous studies (Mann et al. 2012, McKiernan et al. 2012, McKiernan et al. 2014, O'Reilly-Wapstra et al. 2004, 2005a, 2005b, 2007). We detected comparable concentrations of total phenolics within the fine roots of eucalypt species. For example, we found 62.5 mg g^{-1} DM total phenolics in the roots of *E. globulus*, while previous foliage studies reported concentrations in the foliage range from 39.9-210.5 mg g^{-1} DM, varying with tree age, population and treatment (O'Reilly-Wapstra et al. 2007; O'Reilly-Wapstra et al. 2005a; O'Reilly-Wapstra et al. 2005b). We detected the FPCs sideroxylonal A, C and macrocarpal A in the fine roots of eucalypt species and the concentrations of these compounds approached or were equal to those found in foliage (O'Reilly-Wapstra et al. 2004; O'Reilly-Wapstra et al. 2005a; O'Reilly-Wapstra et al. 2005b). Interestingly, FPCs were almost absent in the roots of all subgenus *Eucalyptus* species. These findings are in agreement with patterns observed in eucalypt foliage, where Eschler et al. (2000) found that subgenus *Eucalyptus* species lacked many groups of FPCs. Moreover, we only detected very small to trace amounts of terpenes

within the fine roots of eucalypt species, with higher concentrations of all but two compounds in subgenus *Symphyomyrtus* species. This contrasts with terpenes detected in foliage, where a broad survey of the Tasmanian eucalypt species found that terpenes may form up to 5% of dry mass (Li et al. 1995; 1996), indicating that they may perform more important roles within the foliage of eucalypts. However, the possibility that the extraction method was less effective at extracting terpenes from roots cannot be dismissed.

As the dominant root secondary compounds, phenolics may mediate a range of belowground biotic interactions, including susceptibility to root herbivory and fungal pathogens (Baetz and Martinoia 2014; Erb et al. 2013). Few studies have investigated the role of root phenolics in mediating plant-herbivore interactions (Erb et al. 2013). Surprisingly, total phenolics have been shown to correlate positively with the performance of vine weevils (*Otiorhynchus sulcatus*) and their larvae (Clark et al. 2011; Johnson et al. 2011), possibly resulting from total phenolics covarying with beneficial root characteristics. However, specific phenolic compounds have been shown to confer resistance to belowground herbivory in other plant species (Cole 1987; Cole et al. 1993; Stevenson et al. 2009). It is unclear what role FPCs may play within eucalypt roots, but these compounds affect the palatability of eucalypt foliage for both mammal and insect herbivores (Matsuki et al. 2011; Moore et al. 2005; O'Reilly-Wapstra et al. 2004). Thus, these compounds could also influence the performance of belowground herbivores. Phenolic compounds can also confer resistance to soil fungal pathogens (Lanoue et al. 2010; Wurst et al. 2010). For example, phenolic concentrations within eucalypt roots have been linked to resistance to the fungal pathogen *Phytophthora cinnamomi* (Cahill et al. 1993; Jackson et al. 2000). Phenolic compounds within eucalypt roots could also have important afterlife effects when roots decompose. There is some evidence to suggest that eucalypt species differ in their ability to influence the quantity of soil organic matter as well as the macronutrient content of soils (Orozco-Aceves et al. 2015; Sayad et al. 2012). Interspecific variation in root condensed tannins may contribute to this variation by impacting rates of decomposition and thus the quantity and quality of organic matter in soil (Coq et al. 2010; Wardle et al. 2002). In addition, condensed tannins may influence soil microbial communities and N mineralisation (Schweitzer et al. 2008).

We found that root secondary compounds, especially phenolics, displayed significant phylogenetic signal. There is some evidence to suggest that phylogenetic signal in belowground chemical defence may influence susceptibility to plant enemies. For example, toxic cardenolides found within the roots of milkweeds have been shown to display phylogenetic signal (Vannette and Rasmann 2012) and confer resistance to red milkweed beetles (Rasmann and Agrawal 2011). Similarly, phylogenetic patterns in phenolics among eucalypt species could relate to susceptibility to belowground enemies. For example, eucalypt species belonging to subgenus *Symphyomyrtus* are generally resistant to *P. cinnamomi*, whereas subgenus *Eucalyptus* species have been found to be more susceptible (Podger and Batini 1971; Tippett et al. 1985). Resistence to the pathogen has been related to greater concentrations of total soluble phenolics (Folin-Ciocalteu method) within roots (Cahill et al. 1993; Jackson et al. 2000). This would suggest that as a resistant lineage, subgenus *Symphyomyrtus* would contain greater concentrations of total phenolics within their roots, but this was not the case. We did, however, find that the fine roots of subgenus *Symphyomyrtus* species generally contained greater concentrations of condensed tannins than subgenus *Eucalyptus* species and contained FPCs, which may potentially confer the observed resistance. The fine roots of subgenus *Eucalyptus* species were generally depauperate in condensed tannins and FPCs, potentially leading to greater susceptibility.

In conclusion, our study provides further support for the use of evolutionary history in predicting ecologically relevant plant traits. We show that shared evolutionary history can explain variation in belowground chemical traits within the genus *Eucalyptus*. A recent study indicates that foliar chemicals may also display such phylogenetic signal (Potts et al. 2016). Thus, future studies should test whether phylogenetic patterns in eucalypt root and foliar chemistry are similar, to shed light on any differential gene expression across tissues. The identification of phylogenetic signals in root chemistry may provide a framework for understanding how evolution in plant traits may extend to influence plant ecological interactions, representing an important future avenue of research. This has practical implications for predicting plant susceptibility to belowground pests and pathogens as well as variation in ecosystem processes. Thus, a better understanding of root secondary compounds and their evolution warrants more attention.

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Chapter 6
General discussion

This thesis provides the first evidence that forest tree species can both condition soil microbial communities and respond to this conditioning depending on their evolutionary history. These findings are important because the influences of plant belowground interactions on plant community structure and dynamics are still largely underexplored. Only recently have studies began to use plant evolutionary history to predict plant-soil feedbacks (Anacker et al. 2014; Liu et al. 2012; Münzbergová and Šurinová 2015). Although such studies have found phylogenetic signal in the direction and magnitude of plant-soil feedbacks, and microbes are often implicated as the drivers of such signals, no studies have shown that microbial conditioning can exhibit a phylogenetic signal and drive feedbacks. Using Tasmanian *Eucalyptus* species, I show that evolutionary history can shape soil fungal communities, which may in turn drive phylogenetic signal in plant-soil feedbacks. These findings suggest that closely related species both condition soil communities and respond to this conditioning similarly, highlighting a potential mechanism for phylogenetic structure in plant communities. Further, a functional basis to this phylogenetic feedback is provided by the demonstration of significant phylogenetic signal underlying variation in root defensive chemistry.

Plant-soil feedback can display a phylogenetic signal

Plant-soil feedbacks in the Tasmanian eucalypts were dependent on both the phylogenetic distance between focal and conditioning species and the evolutionary history of the focal species themselves. I am aware of only a single study that has detected phylogenetic signal in the direction and magnitude of plant-soil feedbacks among species, where Anacker et al. (2014) found experimental evidence of a significant phylogenetic signal in whole-soil feedbacks (heterospecific versus conspecific soils) within a Canadian old field community containing 57 species. However, co-occurring species can condition soils variably (Kardol et al. 2007; Perkins and Nowak 2012), and thus, by comparing species' performances in conspecific soils to their average performance across all heterospecific soils, Anacker et al. (2014) may have potentially ignored important variation in their

responses to individual heterospecific soils. Studies suggest that phylogenetic distance between focal and conditioning species can explain variable feedbacks to the soils of co-occurring species (Liu et al. 2012; Münzbergová and Šurinová 2015). Indeed, the degree of phylogenetic distance between focal and conditioning species significantly influenced eucalypt plant-soil feedbacks. The importance of this effect varied among species and was dependent of evolutionary history. For instance, subgenus *Eucalyptus* species typically displayed negative plant-soil feedbacks, where seedling performance increased with increasing phylogenetic distance to the conditioning species (i.e., phylogenetic Janzen-Connell effect), while subgenus *Symphyomyrtus* either showed neutral or small negative responses. These findings provide further evidence that plant-soil feedbacks can display a phylogenetic signal and show that feedbacks may also be dependent on the phylogenetic distance between focal and conditioning species.

Phylogenetic signal in microbial conditioning is associated with plant-soil feedbacks

Closely related eucalypt species assembled similar soil microbial communities, providing a clear candidate mechanism for the phylogenetic patterns in plant-soil feedbacks. Recent studies show that plant evolutionary history can predict some fraction of soil microbial communities, at least under field conditions (Burns et al. 2015; Lugo et al. 2015; Tedersoo et al. 2013). However, such studies may be vulnerable to the confounding effects of environmental variation. For instance, closely related species can occupy similar niches (Baldeck et al. 2013), possibly containing similar microbial communities. Further, spatial variation in soil microbial communities can influence the establishment of plant species (Reinhart et al. 2003). Only a single study has removed such confounding effects. In a replicated and randomised phytoremediation experiment, Bell et al. (2014) found that the abundance of Pezizomycete fungi in the rhizosphere of 11 willow species was directly related to tree evolutionary history. The findings of this thesis provide further evidence for phylogenetic signal in microbial conditioning by showing that eucalypt subgenera can condition distinct fungal communities after a relatively short timeframe of just two years.

Variable plant-soil feedbacks among eucalypt species were related to specific fungal taxa. While many observations of plant-soil feedbacks have been generally

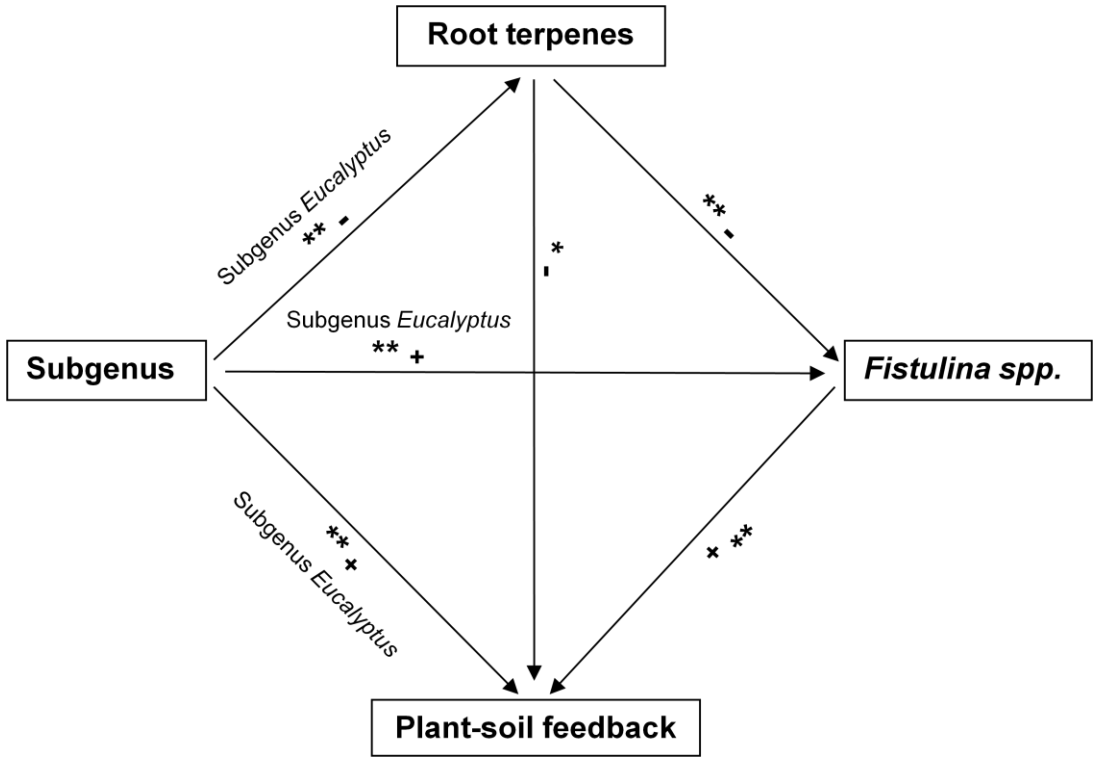
associated with species-specific relationships with fungal pathogens or arbuscular mycorrhizal fungi (Bever 2002; Klironomos 2002; Van der Putten et al. 2007), few studies have sought to identify more specific groups driving feedback responses. For instance, Packer and Clay (2000) identified *Pythium spp.* as the driver of negative plant-soil feedback in black cherry under field conditions. In an experimental setting, I identified several fungal families in conditioned soils that were related to variable plant-soil feedbacks among eucalypt species. The most important of these was *Fistulina spp*., a group known to cause rot of the major roots of several subgenus *Eucalyptus* species (Keane et al. 2000), which provided a strong candidate driver of the phylogenetic signal in plant-soil feedbacks. For instance, subgenus *Eucalyptus* soils generally contained *Fistulina spp*. and experienced negative feedbacks, while subgenus *Symphyomyrtus* species in most cases did not contain this pathogen and exhibited neutral or positive feedbacks. These findings indicate that soil microbes may not just exhibit species-specific relationships, but also similar relationships with close relatives, potentially driving phylogenetic signal in feedbacks.

Phylogenetically conserved traits drive microbial conditioning

Significant relationships were detected between the concentrations of several root secondary metabolites and fungal families that were related to subgeneric differences in feedbacks. Phylogenetic signal in plant chemical traits has been implicated as an underlying driver of plant susceptibility to aboveground herbivores and pathogens (Carrillo-Gavilán et al. 2015; Johnson et al. 2014; Pearse and Hipp 2009), but the traits driving signals in plant-microbial interactions remain unclear. I detected a significant phylogenetic signal in several groups of root secondary metabolites that are known to influence soil microbial communities (Baetz and Martinoia 2014; Ehrenfeld et al. 2005), including terpenes and phenolics. Indeed, variation in terpene concentrations, but not other compounds (Appendix C, Table C1 and Box 1), was significantly correlated with the presence of *Fistulina spp*. in conditioned soils, which were the best candidate for explaining negative feedbacks in subgenus *Eucalyptus*. For instance, subgenus *Eucalyptus* species displayed significantly lower concentrations of terpenes in their roots and contained *Fistulina spp*. in their soils, while subgenus *Symphyomyrtus* species displayed higher concentrations and generally lacked *Fistulina spp*. in their soils. Indeed, these compounds have been shown to exhibit antimicrobial properties (Gilles et al. 2010). The influence of

terpenes on *Fistulina spp*. and *Fistulina spp*. on feedbacks was further evidenced by a significant relationship between terpene concentrations and plant-soil feedbacks. This suggests that susceptibility to soil pathogens and thus, negative feedbacks, may be related to root chemical traits, although there are likely other chemical differences between these subgenera which could also be implicated.

Box 1. A summary of the potential mechanisms driving plant-soil feedback in the eucalypt species studied.



Arrows represent non-parametric Kendall's rank correlations between factors, signs (+ or -) indicate the direction of relationships and stars represent statistical significance, where * P = < 0.05 and ** P = < 0.01. Correlations examined species-level data from chapters 3, 4 and 5 in R using the Kendall function from the Package *Kendall*. Plant-soil feedback values are the slopes of the linear relationships between the relative responses (total biomass) of eucalypt species to conditioned soils and phylogenetic distance between focal and conditioning species. Positive values represent negative plant-soil feedback, or a phylogenetic Janzen-Connell effect, while negative values represent positive feedback. Direct relationships involving subgenus were tested using two-tailed tests, whereas other relationships were tested with one-tailed tests under the following specific hypotheses: (i) increasing terpene concentrations would be associated with the absence of the fungal pathogen *Fistulina spp.* in conditioned soils, (ii) increasing terpene concentrations would be associated with feedback slopes becoming negative (i.e., positive feedback) and (iii) the presence of *Fistulina spp.* would be associated with feedback slopes becoming positive (i.e., negative feedback). Subgenus significantly influenced the direction and magnitude of plant-soil feedbacks among eucalypt species. This phylogenetic signal may have occurred through subgenus *Eucalyptus*, exhibiting lower concentrations of anti-microbial terpenes, and thus, increasing susceptibility to *Fistulina spp.*, which may have in turn reduced the performance of con-generic seedlings in subgenus *Eucalyptus* conditioned soils.

Phylogenetic plant-soil feedbacks may drive vegetation structure and dynamics

Most of the significant feedback responses detected were consistent with a phylogenetic Janzen-Connell effect (Liu et al. 2012). There is evidence to suggest that forest communities may be phylogenetically structured, where neighbouring tree species are less phylogenetically related than expected by chance (Liu et al. 2012; Zhu et al. 2015). Experimental evidence suggests that such patterns are driven by a phylogenetic Janzen-Connell effect, where the performance of seedlings in conditioned soils is dependent upon the degree of phylogenetic relatedness between focal and conditioning species (Liu et al. 2012). The findings of this thesis provide further support for the phylogenetic Janzen-Connell effect and its novel finding is that susceptibility to this effect displays phylogenetic signal itself. Subgenus *Eucalyptus* species typically displayed trends consistent with the phylogenetic Janzen-Connell effect, while subg. *Symphyomyrtus* species typically did not. This is the first experimental evidence that microbial conditioning and feedback to this conditioning occurs in eucalypts, with potentially important consequences for the structure and dynamics of eucalypt dominated ecosystems. For instance, such subgeneric differences in plant-soil feedbacks could play a role in the structure of mixed eucalypt stands containing species belonging to each subgenus (Austin et al. 1983; Davidson and Reid 1980; Duff et al. 1983). Indeed, the findings of chapter 1 provide evidence that plant-soil feedbacks may operate in such stands, where *E. globulus* (subgenus *Symphyomyrtus*) responded significantly to inoculation with native soils in a manner that was constistent with positive plant-soil feedback, while *E. obliqua* (subgenus *Eucalyptus*) exhibited responses that were consistent with negative plant-soil feedback, although statistically insignificant. However, feedbacks were dependent on whether inoculum was collected from unburnt or burnt stands, suggesting that forest fire may disrupt plant-soil feedbacks. Variable plant-soil feedbacks among eucalypt species could also contribute to the relative abundance or rarity of eucalypt species (Mangan et al. 2010) or even their successional status (Kardol et al. 2006), as shown in other systems. As the dominant genus in Australia, *Eucalyptus* provides habitat for a range of dependent organisms and its species form the foundation for a range of ecosystems. Thus, the investigation of plant-soil feedbacks in the genus as well as the interactive effects of external factors, such as fire, warrants further research.

Implications for phylogenetic ecology

Using several integrated experiments, this thesis shows important mechanistic linkages between plant evolutionary history and ecology and that these linkages are dependent on taxonomic scale. While several studies have shown that the evolutionary history of species can shape ecological interactions (e.g., Anacker et al. 2014; Gilbert et al. 2015; Liu et al. 2012; Vannette and Rasmann 2012), this thesis provides some of the first mechanistic evidence linking evolutionary history and ecological patterns. Subgenus *Eucalyptus*, but not *Symphyomyrtus*, generally exhibited significant negative plant-soil feedback. This phylogenetic signal was related to distinct fungal communtites and root chemistry between the eucalypt subgenera (see Box 1). Because these experiments were taxonomically focussed (i.e., just one genus with dense species sampling), I also show that there was a clear step change in when relatedness matters as well as the direction of its effect. For instance, phylogenetic relatedness was an important factor influencing plant-soil feedbacks across, but not within, subgenera and the direction of responses varied from positive in subgenus *Eucalyptus* to negative or neutral in *Symphyomyrtus*. Such changes may explain why metanalyses and studies analysing for the importance of phylogenetic distance across broad groups of species have found little to no effect (Mehrabi et al. 2015; Mehrabi and Tuck 2015). These findings highlight the need for future studies linking ecology and evolution across a range of taxonomic scales.

Concluding remarks

The findings of my thesis provide further support for the use of evolutionary history as a predictor of plant-ecological interactions. Evolutionary relationships among plant species can predict a range of aboveground ecological interactions including, susceptibility to herbivores and pathogens (Gilbert et al. 2015; Hill and Kotanen 2011; Potts et al. 2016). By showing a phylogenetic signal in microbial conditioning and plant-soil feedbacks, I argue that evolutionary history can similarly predict plant belowground ecological interactions. These phylogenetic signals suggest that tree species condition soil microbial communities and respond to the soils of co-occurring species depending on their evolutionary history. Such phylogenetic signals may have practical implications for planning restoration plantings (Schweizer et al. 2013) or predicting the invasiveness of exotic species (Strauss et al. 2006). With continuing advances in genetic technologies, the microbiome is becoming

increasingly accessible for research and how it interacts with the aboveground world represents a major frontier in ecological research. The further elucidation of the linkages between ecology and evolution may simplify our understanding of the manner in which plant communities are structured and how such communities will respond to species loss or invasions in a changing world.

References

Agrawal AA, Fishbein M, Jetter R, Salminen JP, Goldstein JB, Freitag AE, Sparks JP (2009a) Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias spp.*): chemistry, ecophysiology, and insect behavior. New Phytol 183: 848-867.

Agrawal AA, Salminen JP, Fishbein M (2009b) Phylogenetic trends in phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation. Evolution 63: 663-673.

Allen EB, Allen MF, Egerton-Warburton L, Corkidi L, Gómez-Pompa A (2003) Impacts of early-and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. Ecol Appl 13: 1701-1717.

Allen MF, Allen EB, Gómez-Pompa A (2005) Effects of mycorrhizae and nontarget organisms on restoration of a seasonal tropical forest in Quintana Roo, Mexico: factors limiting tree establishment. Restor Ecol 13: 325-333.

Anacker BL, Klironomos JN, Maherali H, Reinhart KO, Strauss SY (2014) Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. Ecol Lett 17: 1613-1621.

Anderson IC, Barbara D, Kerry K, Oula G, Chambers SM, Tissue DT, Cairney JW (2013) Interactive effects of preindustrial, current and future atmospheric CO_2 concentrations and temperature on soil fungi associated with two *Eucalyptus* species. FEMS Microbiol Ecol 83: 425-437.

Ashton D, Attiwill P (1994) Tall open-forests. In: R Groves (ed) Australian vegetation. Cambridge University Press, Cambridge.

Austin M, Cunningham R, Wood J (1983) The subgeneric composition of eucalypt forest stands in a region of south-eastern Australia. Aust J Bot 31: 63-71.

Baetz U, Martinoia E (2014) Root exudates: the hidden part of plant defense. Trends Plant Sci 19: 90-98.

Bagchi R, Gallery RE, Gripenberg S, Gurr SJ, Narayan L, Addis CE, Freckleton RP, Lewis OT (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506: 85-88.

Baker SC, Read SM (2011) Variable retention silviculture in Tasmania's wet forests: ecological rationale, adaptive management and synthesis of biodiversity benefits. Aust For 74: 218-232.

Baldeck CA, Kembel SW, Harms KE, Yavitt JB, John R, Turner BL, Chuyong GB, Kenfack D, Thomas DW, Madawala S (2013) A taxonomic comparison of local habitat niches of tropical trees. Oecologia 173: 1491-1498.

Bates D, Maechler M, Bolker B, Walker S (2015) lme4: Linear mixed-effects models using Eigen and S4. R package version 11-10.

Baxendale C, Orwin KH, Poly F, Pommier T, Bardgett RD (2014) Are plant–soil feedback responses explained by plant traits? New Phytol 204: 408-423.

Bell TH, Hassan SE-D, Lauron-Moreau A, Al-Otaibi F, Hijri M, Yergeau E, St-Arnaud M (2014) Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. The ISME journal 8: 331-343.

Bever JD (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant Soil 244: 281-290.

Bezemer T, Lawson CS, Hedlund K, Edwards AR, Brook AJ, Igual JM, Mortimer SR, Van Der Putten WH (2006) Plant species and functional group effects on abiotic and microbial soil properties and plant–soil feedback responses in two grasslands. J Ecol 94: 893-904.

Blomberg SP and Garland Jr T (2002) Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. J. Evol. Biol. 15:899-910.

Blomberg SP, Garland Jr T, Ives AR, Crespi B (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57: 717-745.

Bond WJ, Van Wilgen BW (2012) Fire and plants. Springer Science & Business Media.

Borzak CL, O'Reilly-Wapstra JM, Potts BM (2015) Direct and indirect effects of marsupial browsing on a foundation tree species. Oikos 124: 515-524.

Brinkman PE, Van der Putten WH, Bakker E-J, Verhoeven KJ (2010) Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. J Ecol 98: 1063-1073.

Brooker MIH (2000) A new classification of the genus *Eucalyptus* L'Her.(Myrtaceae). Aust Syst Bot 13: 79-148.

Burdon JJ, Thrall PH, Ericson, Lars (2006) The current and future dynamics of disease in plant communities. Annu Rev Phytopathol 44: 19-39.

Burns JH, Anacker BL, Strauss SY, Burke DJ (2015) Soil microbial community variation correlates most strongly with plant species identity, followed by soil chemistry, spatial location and plant genus. AoB plants 7.

Cahill D, Bennett I, McComb J (1993) Mechanisms of resistance to *Phytophthora cinnamomi* in clonal, micropropagated *Eucalyptus marginata*. Plant Pathol 42: 865-872.

Cai Y, Gaffney SH, Lilley TH, Haslam E (1989) Carbohydrate-polyphenol complexation. In: Hemingway RW, Karchesy JJ (eds) Chemistry and significance of condensed tannins, Plenum, New York.

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427: 731-733.

Carrillo-Gavilán A, Moreira X, Zas R, Gonzalez-Voyer A, Vilà M, Sampedro L (2015) Phylogenetic and biogeographical patterns in defensive strategies and quantitative allocation to chemical defences in Palaearctic and Nearctic pine trees. J Biogeogr 42: 684-693.

Carson WP, Root RB (2000) Herbivory and plant species coexistence: community regulation by an outbreaking phytophagous insect. Ecol Monogr 70: 73-99.

Certini G (2005) Effects of fire on properties of forest soils: a review. Oecologia 143: 1-10.

Chapman SK, Langley JA, Hart SC, Koch GW (2006) Plants actively control nitrogen cycling: uncorking the microbial bottleneck. New Phytol 169: 27-34.

Chen YL, Kang LH, Dell B (2006) Inoculation of *Eucalyptus urophylla* with spores of *Scleroderma* in a nursery in south China: comparison of field soil and potting mix. For Ecol Manage 222: 439-449.

Claridge AW (2002) Ecological role of hypogeous ectomycorrhizal fungi in Australian forests and woodlands. Diversity and Integration in Mycorrhizas. Springer.

Clark KE, Hartley SE, Johnson SN (2011) Does mother know best? The preference–performance hypothesis and parent–offspring conflict in aboveground–belowground herbivore life cycles. Ecol Entomol 36: 117-124.

Cole RA (1987) Intensity of radicle fluorescence as related to the resistance of seedlings of lettuce to the lettuce root aphid and carrot to the carrot fly. Ann Appl Biol 111: 629-639.

Cole RA, Riggall W, Morgan A (1993) Electronically monitored feeding behaviour of the lettuce root aphid (*Pemphigus bursarius*) on resistant and susceptible lettuce varieties. Entomol Exp Appl 68: 179-185.

Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: PJd Boer, G Gradwell (eds) Dynamics of Numbers in Populations. Center for Agricultural Publication and Documentation, Wageningen.

Coq S, Souquet J-M, Meudec E, Cheynier V, Hättenschwiler S (2010) Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. Ecology 91: 2080-2091.

Crisp MD, Cook LG (2012) Phylogenetic niche conservatism: what are the underlying evolutionary and ecological causes? New Phytol 196: 681-694.

Davidson N, Reid J (1980) Comparison of the early growth characteristics of the *Eucalyptus* subgenera *Monocalyptus* and *Symphyomyrtus*. Aust J Bot 28: 453-461.

de Vries FT, Manning P, Tallowin JRB, Mortimer SR, Pilgrim ES, Harrison KA, Hobbs PJ, Quirk H, Shipley B, Cornelissen JHC (2012) Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol Lett 15: 1230–1239.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72: 5069-5072.

Dooley SR, Treseder KK (2012) The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109: 49-61.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7: 214.

Duff G, Reid J, Jackson W (1983) The occurrence of mixed stands of the *Eucalyptus* subgenera *Monocalyptus* and *Symphyomyrtus* in south-eastern Tasmania. Aust J Bot 8: 405-414.

Dungey HS, Potts BM, Whitham TG, Li HF (2000) Plant genetics affects arthropod community richness and composition: evidence from a synthetic eucalypt hybrid population. Evolution 54: 1938-1946.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460-2461.

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant-soil system. Annu Rev Environ Resour 30: 75-115.

Ellis R, Pennington P (1992) Factors affecting the growth of *Eucalyptus delegatensis* seedlings in inhibitory forest and grassland soils. Plant Soil 145: 93-105.

Erb M, Huber M, Robert CA, Ferrieri AP, Machado RA, Arce CC (2013) The role of plant primary and secondary metabolites in root-herbivore behaviour, nutrition and physiology. Adv Insect Physiol 45: 53-95.

Eschler B, Pass D, Willis R, Foley W (2000) Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. Biochem Syst Ecol 28: 813-824.

Eyles A, Davies NW, Mohammed C (2003) Novel detection of formylated phloroglucinol compounds (FPCs) in the wound wood of *Eucalyptus globulus* and *E. nitens*. J Chem Ecol 29: 881-898.

Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu W, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao J, Cornelissen JH (2013) Linking litter decomposition of above-and below-ground organs to plant–soil feedbacks worldwide. J Ecol 101: 943-952.

Gilbert GS, Briggs HM, Magarey R (2015) The impact of plant enemies shows a phylogenetic signal. PloS One 10: e0123758.

Gilbert GS, Magarey R, Suiter K, Webb CO (2012) Evolutionary tools for phytosanitary risk analysis: phylogenetic signal as a predictor of host range of plant pests and pathogens. Evol appl 5: 869-878.

Gilbert GS, Webb CO (2007) Phylogenetic signal in plant pathogen–host range. Proc Natl Acad Sci 104: 4979-4983.

Gill AM (1997) Eucalypts and fires: interdependent or independent. In: A Williams, J Woinarski (eds) Eucalypt ecology: individuals to ecosystems. Cambridge University Press, Cambridge, UK.

Gilles M, Zhao J, An M, Agboola S (2010) Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. Food Chem 119: 731-737.

Gleadow RM, Veechies AC, Woodrow IE (2003) Cyanogenic *Eucalyptus nobilis* is polymorphic for both prunasin and specific beta-glucosidases. Phytochemistry 63: 699-704. doi: 10.1016/s0031-9422(03)00245-0.

Graham HD (1992) Stabilization of the Prussian blue color in the determination of polyphenols. J Agric Food Chem 40: 801-805.

Griffin A, Burgess I, Wolf L (1988) Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'hérit. - a Review. Aust J Bot 36: 41-66.

Hagerman AE, Butler LG (1980) Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 28: 947-952.

Harvest T, Davidson N, Close D (2008) Is decline in high altitude eucalypt forests related to rainforest understorey development and altered soil bacteria following the long absence of fire? Austral Ecol 33: 880-890.

Harvey PH, Pagel MD (1991) The comparative method in evolutionary biology. Oxford, UK: Oxford University Press.

Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst: 191-218.

Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. Ecology 80: 1150-1156.

Hill SB, Kotanen PM (2011) Phylogenetic structure predicts capitular damage to Asteraceae better than origin or phylogenetic distance to natives. Oecologia 166: 843-851.

Horner-Devine CM, Leibold MA, Smith VH, Bohannan BJ (2003) Bacterial diversity patterns along a gradient of primary productivity. Ecol Lett 6: 613-622.

Hosaka K, Bates ST, Beever RE, Castellano MA, Colgan W, Domínguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR (2006) Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. Mycologia 98: 949-959.

Howe H, Brown J, Zorn-Arnold B (2002) A rodent plague on prairie diversity. Ecol Lett 5: 30-36.

Humphreys F, Lambert MJ (1965) An examination of a forest site which has exhibited the ash-bed effect. Soil Res 3: 81-94.

Humphreys J, O'Reilly-Wapstra J, Harbard J, Davies N, Griffin A, Jordan G, Potts B (2008) Discrimination between seedlings of *Eucalyptus globulus*, *E. nitens* and their F1 hybrid using near-infrared reflectance spectroscopy and foliar oil content. Silvae Genet 57: 4-5.

Ives AR, Midford PE, Garland T (2007) Within-species variation and measurement error in phylogenetic comparative methods. Syst Biol 56: 252-270.

Jackson T, Burgess T, Colquhoun I, Hardy GS (2000) Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. Plant Pathol 49: 147-154.

Janzen DH (1970) Herbivores and the number of tree species in tropical forests. Am Nat 104: 501-528.

Johnson DJ, Beaulieu WT, Bever JD, Clay K (2012) Conspecific negative density dependence and forest diversity. Science 336: 904-907.

Johnson MT, Ives AR, Ahern J, Salminen JP (2014) Macroevolution of plant defenses against herbivores in the evening primroses. New Phytol 203: 267-279.

Johnson SN, Barton AT, Clark KE, Gregory PJ, McMenemy LS, Hancock RD (2011) Elevated atmospheric carbon dioxide impairs the performance of root-feeding vine weevils by modifying root growth and secondary metabolites. Global Change Biol 17: 688-695.

Jones R, McKinnon G, Potts B, Vaillancourt R (2005) Genetic diversity and mating system of an endangered tree *Eucalyptus morrisbyi*. Aust J Bot 53: 367-377.

Kamgan Nkuekam G, Wingfield MJ, Roux J (2013) *Ceratocystis* species, including two new taxa, from *Eucalyptus* trees in South Africa. Aust Plant Path 42: 283-311.

Kardol P, Cornips NJ, van Kempen MM, Bakx-Schotman JT, van der Putten WH (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77: 147-162.

Kardol P, Martijn Bezemer T, van der Putten WH (2006) Temporal variation in plant–soil feedback controls succession. Ecol Lett 9: 1080-1088.

Keane P, Kile G, Podger F, Brown B (2000) Diseases and pathogens of eucalypts. CSIRO Publishing, Collingwood.

Keeley JE, Fotheringham C (2000) Role of fire in regeneration from seed. In: M Fenner (ed) Seeds: the ecology of regeneration in plant communities. CABI Publishing, Brisbane.

Kier G, Mutke J, Dinerstein E, Ricketts TH, Küper W, Kreft H, Barthlott W (2005) Global patterns of plant diversity and floristic knowledge. J Biogeogr 32: 1107-1116.

Kile G, Hardy R, Turnbull C (1979) The association between *Abantiades latipennis* (*Lepidoptera*, family Hepialidae) and *Eucalyptus obliqua* and *Eucalyptus regnans* in Tasmania. Aust J Entomol 18: 7-17.

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67-70.

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166: 1063-1068.

Kraft NJ, Ackerly DD (2010) Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. Ecol Monogr 80: 401-422.

Kulmatiski A, Anderson-Smith A, Beard KH, Doucette-Riise S, Mazzacavallo M, Nolan NE, Ramirez RA, Stevens JR (2014) Most soil trophic guilds increase plant growth: a meta-analytical review. Oikos 123: 1409-1419.

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant–soil feedbacks: a meta-analytical review. Ecol Lett 11: 980-992.

Kulmatiski A, Kardol P (2008) Getting plant-soil feedbacks out of the greenhouse: experimental and conceptual approaches. In: K Esser, U Lüttige, W Beyschlag, J Murata (eds) Progress in Botany. Springer.

Kuznetsova A, Brockhoff P, Christensen R (2015) lmerTest: Tests in Linear Mixed Effects Models. R package version 20–29.

Lanoue A, Burlat V, Henkes GJ, Koch I, Schurr U, Röse US (2010) De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. New Phytol 185: 577-588.

Lawler I, Foley W, Eschler B, Pass D, Handasyde K (1998) Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. Oecologia 116: 160-169.

Leake J, Johnson D, Donnelly D, Muckle G, Boddy L, Read D (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant

communities and agroecosystem functioning. Canadian Journal of Botany 82: 1016-1045.

Lejon DP, Chaussod R, Ranger J, Ranjard L (2005) Microbial community structure and density under different tree species in an acid forest soil (Morvan, France). Microb Ecol 50: 614-625.

Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. Syst Biol 50: 913-925.

Li H, Madden J (1995) Analysis of leaf oils from a *Eucalyptus* species trial. Biochem Syst Ecol 23: 167-177.

Li H, Madden J, Potts B (1995) Variation in volatile leaf oils of the Tasmanian *Eucalyptus* species - I. Subgenus *Monocalyptus*. Biochem Syst Ecol 23: 299-318.

Li H, Madden J, Potts B (1996) Variation in leaf waxes of the Tasmanian *Eucalyptus* species - II. Subgenus *Symphyomyrtus*. Biochem Syst Ecol 25: 631-657.

Ling-Lee M, Chilvers G, Ashford A (1977) A histochemical study of phenolic materials in mycorrhizal and uninfected roots of *Eucalyptus fastigata* Deane and Maiden. New Phytol 78: 313-328.

Liu X, Liang M, Etienne RS, Wang Y, Staehelin C, Yu S (2012) Experimental evidence for a phylogenetic Janzen–Connell effect in a subtropical forest. Ecol Lett 15: 111-118.

Loneragan O, Loneragan J (1964) Ashbed and nutrients in the growth of seedlings of Karri (*Eucalyptus diversicolor* FvM). J R Soc West Aust 47: 75-80.

Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol Lett 11: 995-1003.

Lu X, Malajczuk N, Dell B (1998) Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi. Mycorrhiza 8: 81-86.

Lugo MA, Reinhart KO, Menoyo E, Crespo EM, Urcelay C (2015) Plant functional traits and phylogenetic relatedness explain variation in associations with root fungal endophytes in an extreme arid environment. Mycorrhiza 25: 85-95.

Mangan SA, Schnitzer SA, Herre EA, Mack KM, Valencia MC, Sanchez EI, Bever JD (2010) Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466: 752-755.

Mann AN, O'Reilly-Wapstra J, Iason G, Sanson G, Davies NW, Tilyard P, Williams D, Potts B (2012) Mammalian herbivores reveal marked genetic divergence among populations of an endangered plant species. Oikos 121: 268-276.

Manning P, Morrison S, Bonkowski M, Bardgett RD (2008) Nitrogen enrichment modifies plant community structure via changes to plant–soil feedback. Oecologia 157: 661-673.

Maron JL, Klironomos J, Waller L, Callaway RM (2014) Invasive plants escape from suppressive soil biota at regional scales. J Ecol 102: 19-27.

Matsuki M, Foley WJ, Floyd RB (2011) Role of volatile and non-volatile plant secondary metabolites in host tree selection by Christmas beetles. J Chem Ecol 37: 286-300.

McCarthy-Neumann S, Kobe RK (2010a) Conspecific and heterospecific plant-soil feedbacks influence survivorship and growth of temperate tree seedlings. J Ecol 98: 408-418.

McCarthy-Neumann S, Kobe RK (2010b) Conspecific plant-soil feedbacks reduce survivorship and growth of tropical tree seedlings. J Ecol 98: 396-407.

McDowell NG, Coops NC, Beck PS, Chambers JQ, Gangodagamage C, Hicke JA, Huang C-y, Kennedy R, Krofcheck DJ, Litvak M (2015) Global satellite monitoring of climate-induced vegetation disturbances. Trends Plant Sci 20: 114-123.

McKiernan AB, Hovenden MJ, Brodribb TJ, Potts BM, Davies NW, O'Reilly-Wapstra JM (2014) Effect of limited water availability on foliar plant secondary metabolites of two *Eucalyptus* species. Environ Exp Bot 105: 55-64.

McKinnon GE, Vaillancourt RE, Steane DA, Potts BM (2008) An AFLP marker approach to lower-level systematics in *Eucalyptus* (Myrtaceae). Am J Bot 95: 368-380.

Mehrabi Z, Bell T, Lewis OT (2015) Plant-soil feedbacks from 30-year family-specific soil cultures: phylogeny, soil chemistry and plant life stage. Ecology and Evolution 5: 2333-2339. doi: 10.1002/ece3.1487.

Mehrabi Z, Tuck SL (2015) Relatedness is a poor predictor of negative plant–soil feedbacks. New Phytol 205: 1071-1075.

Meisner A, de Boer W, Verhoeven KJ, Boschker HT, van der Putten WH (2011) Comparison of nutrient acquisition in exotic plant species and congeneric natives. J Ecol 99: 1308-1315.

Moore BD, Foley WJ (2005) Tree use by koalas in a chemically complex landscape. Nature 435: 488-490.

Moore BD, Foley WJ, Wallis IR, Cowling A, Handasyde KA (2005) *Eucalyptus* foliar chemistry explains selective feeding by koalas. Biol Lett 1: 64-67.

Münzbergová Z, Šurinová M (2015) The importance of species phylogenetic relationships and species traits for the intensity of plant-soil feedback. Ecosphere 6: Article 234.

Mutabaruka R, Hairiah K, Cadisch G (2007) Microbial degradation of hydrolysable and condensed tannin polyphenol–protein complexes in soils from different land-use histories. Soil Biol Biochem 39: 1479-1492.

Nakadai R, Murakami M, Hirao T (2014) Effects of phylogeny, leaf traits, and the altitudinal distribution of host plants on herbivore assemblages on congeneric *Acer* species. Oecologia 175: 1237-1245.

Neyland M, Hickey J, Beadle C, Bauhus J, Davidson N, Edwards L (2009) An examination of stocking and early growth in the Warra silvicultural systems trial confirms the importance of a burnt seedbed for vigorous regeneration in *Eucalyptus obliqua* forest. For Ecol Manage 258: 481-494.

Neyland M, Wilkinson G, Edwards L (1999) The Forestier silvicultural systems trial: Alternatives to clearfelling. Tas Forests 11: 35-48.

Noble I (1989) Ecological traits of the *Eucalyptus* L'Herit subgenera *Monocalyptus* and *Symphyomyrtus*. Aust J Bot 37: 207-224.

Northup RR, Yu Z, Dahlgren RA, Vogt KA (1995) Polyphenol control of nitrogen release from pine litter. Nature 377: 227-229.

O'Reilly-Wapstra J, McArthur C, Potts B (2004) Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. Funct Ecol 18: 677-684.

O'Reilly-Wapstra JM, Bailey JK, McArthur C, Potts BM (2010) Genetic-and chemical-based resistance to two mammalian herbivores varies across the geographic range of *Eucalyptus globulus*. Evol Ecol Res 12: 491-505.

O'Reilly-Wapstra JM, Humphreys JR, Potts BM (2007) Stability of genetic-based defensive chemistry across life stages in a *Eucalyptus* species. J Chem Ecol 33: 1876-1884.

O'Reilly-Wapstra JM, Potts BM, McArthur C, Davies NW (2005a) Effects of nutrient variability on the genetic-based resistance of *Eucalyptus globulus* to a mammalian herbivore and on plant defensive chemistry. Oecologia 142: 597-605.

O'Reilly-Wapstra JM, Potts BM, Mcarthur C, Davies NW, Tilyard P (2005b) Inheritance of resistance to mammalian herbivores and of plant defensive chemistry in an *Eucalyptus* species. J Chem Ecol 31: 357-375.

Orozco-Aceves M, Standish RJ, Tibbett M (2015) Long-term conditioning of soil by plantation eucalypts and pines does not affect growth of the native jarrah tree. For Ecol Manage 338: 92-99.

Orwin KH, Buckland SM, Johnson D, Turner BL, Smart S, Oakley S, Bardgett RD (2010) Linkages of plant traits to soil properties and the functioning of temperate grassland. J Ecol 98: 1074-1083.

Packer A, Clay K (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404: 278-281.

Page DE, Close D, Beadle CL, Wardlaw TJ, Mohammed CL (2013) Seasonal dynamics in understorey abundance and carbohydrate concentration in

relation to browsing and bark stripping of Tasmanian *Pinus radiata* plantations. For Ecol Manage 296: 98-107.

Paradis E, Blomberg S, Bolker B, Claude J, Cuong HS, Desper R, Didier G, Durand B, Dutheil J, Gascuel O (2012) Package 'ape': Analyses of phylogenetics and evolution. R package Version 34 4.

Pearse IS, Hipp AL (2009) Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. Proc Natl Acad Sci 106: 18097-18102.

Perkins LB, Nowak RS (2012) Soil conditioning and plant–soil feedbacks affect competitive relationships between native and invasive grasses. Plant Ecol 213: 1337-1344.

Petermann JS, Fergus AJ, Turnbull LA, Schmid B (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. Ecology 89: 2399-2406.

Podger F, Batini F (1971) Susceptibility to *Phytophthora cinnamomi* root-rot of thirty six species of *Eucalyptus*. Aust Forest Res 5: 9-20.

Porter LJ, Hrstich LN, Chan BG (1985) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25: 223-230.

Potts BM, Sandhu KS, Wardlaw T, Freeman J, Li H, Tilyard P, Park RF (2016) Evolutionary history shapes the susceptibility of an island tree flora to an exotic pathogen. For Ecol Manage 368: 183-193.

Pregitzer CC, Bailey JK, Hart SC, Schweitzer JA (2010) Soils as agents of selection: feedbacks between plants and soils alter seedling survival and performance. Ecology and Evolution 24: 1045-1059.

Pryor L (1963) Ash bed growth response as a key to plantation establishment on poor sites. Aust For 27: 48-51.

Pryor L, Johnson L (1981) *Eucalyptus*, the universal Australian. In: A Keast (ed) Ecological biogeography of Australia The Hague, Dr. W. Junk.

Pryor LD, Johnson LAS (1971) A classification of the eucalypts. Australian National University, Canberra.

Rafiqpoor D, KIER G, Kreft H (2005) Global centers of vascular plant diversity. Nova Acta Leopoldina NF 92: 61-83.

Rasmann S, Agrawal AA (2008) In defense of roots: a research agenda for studying plant resistance to belowground herbivory. Plant Physiol 146: 875-880.

Rasmann S, Agrawal AA (2011) Evolution of specialization: a phylogenetic study of host range in the red milkweed beetle (*Tetraopes tetraophthalmus*). Am Nat 177: 728-737.

Reinhart KO, Packer A, Van der Putten WH, Clay K (2003) Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. Ecol Lett 6: 1046-1050.

Reinhart KO, Wilson GW, Rinella MJ (2012) Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. Ecol Lett 15: 689-695.

Revell LJ (2012) Phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol 3: 217-223.

Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D, Ripley MB (2015) Package 'MASS'. R package version 73-45.

Rosenzweig ML (1995) Species diversity in space and time. Cambridge University Press.

Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148: 1547-1556.

Sanger JC, Davidson NJ, O'GRADY AP, Close DC (2011) Are the patterns of regeneration in the endangered *Eucalyptus gunnii ssp. divaricata* shifting in response to climate? Austral Ecol 36: 612-620.

Sayad E, Hosseini S, Hosseini V, Salehe-Shooshtari M (2012) Soil macrofauna in relation to soil and leaf litter properties in tree plantations. J For Sci 58: 170-180.

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85: 591-602.

Schloss PD, Gevers D, Westcott SL (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PloS one 6: e27310.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537-7541.

Schradin K, Cipollini D (2012) The sign and strength of plant-soil feedback for the invasive shrub, Lonicera maackii, varies in different soils. Forests 3: 903-922.

Schulze E-D, Mooney HA (2012) Biodiversity and ecosystem function. Springer Science & Business Media.

Schweitzer JA, Bailey JK, Rehill BJ, Martinsen GD, Hart SC, Lindroth RL, Keim P, Whitham TG (2004) Genetically based trait in a dominant tree affects ecosystem processes. Ecol Lett 7: 127-134.

Schweitzer JA, Madritch MD, Bailey JK, LeRoy CJ, Fischer DG, Rehill BJ, Lindroth RL, Hagerman AE, Wooley SC, Hart SC (2008) From genes to ecosystems: the genetic basis of condensed tannins and their role in nutrient regulation in a *Populus* model system. Ecosystems 11: 1005-1020.

Schweizer D, Gilbert GS, Holl KD (2013) Phylogenetic ecology applied to enrichment planting of tropical native tree species. For Ecol Manage 297: 57-66.

Senior J, Schweitzer J, O'Reilly-Wapstra J, Chapman S, Steane D, Langley A, Bailey J (2013) Phylogenetic responses of forest trees to global change. PloS One 8: e60088.

Smith LM, Reynolds HL (2012) Positive plant-soil feedback may drive dominance of a woodland invader, *Euonymus fortunei*. Plant Ecol 213: 853-860.

Smith S, Read S (1997) Mycorrhizal Symbiosis, 2nd edn. Academic Press, San Diego.

Soteras F, Renison D, Becerra AG (2013) Growth response, phosphorus content and root colonization of *Polylepis australis* Bitt. seedlings inoculated with different soil types. New forests 44: 577-589.

Steane DA, Nicolle D, Sansaloni CP, Petroli CD, Carling J, Kilian A, Myburg AA, Grattapaglia D, Vaillancourt RE (2011) Population genetic analysis and phylogeny reconstruction in *Eucalyptus* (Myrtaceae) using high-throughput, genome-wide genotyping. Mol Phylogen Evol 59: 206-224.

Stevenson PC, Muyinza H, Hall DR, Porter EA, Farman DI, Talwana H, Mwanga RO (2009) Chemical basis for resistance in sweetpotato *Ipomoea batatas* to the sweetpotato weevil *Cylas puncticollis*. Pure Appl Chem 81: 141-151.

Stone C, Simpson J, Gittins R (1998) Differential impact of insect herbivores and fungal pathogens on the *Eucalyptus* subgenera *Symphyomyrtus* and *Monocalyptus* and genus *Corymbia*. Aust J Bot 46: 723-734.

Strauss SY, Webb CO, Salamin N (2006) Exotic taxa less related to native species are more invasive. Proc Natl Acad Sci 103: 5841-5845.

Tedersoo L, Mett M, Ishida TA, Bahram M (2013a) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. New Phytol 199: 822-831.

Terborgh J (2012) Enemies maintain hyperdiverse tropical forests. Am Nat 179: 303-314.

Tieszen LL (2012) Vegetation and production ecology of an Alaskan arctic tundra. Springer Science & Business Media.

Tilman D (1988) Plant strategies and the dynamics and structure of plant communities. Princeton University Press.

Tilman D, Pacala S (1993) The maintenance of species richness in plant communities. In: Ricklefs RE, Schluter D (eds) Species diversity in ecological communities: historical and geogrpahical perspectives. University of Chicago Press, pp 13-25.

Tippett J, Hill T, Shearer B (1985) Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi*. Aust J Bot 33: 409-418.

Trocha LK, Kałucka I, Stasińska M, Nowak W, Dabert M, Leski T, Rudawska M, Oleksyn J (2012) Ectomycorrhizal fungal communities of native and non-native *Pinus* and *Quercus* species in a common garden of 35-year-old trees. Mycorrhiza 22: 121-134.

van Dam NM (2009) Belowground herbivory and plant defenses. Annu Rev Ecol Evol Syst 40: 373-391.

Van der Heijden MG, Bardgett RD, van Straalen NM (2008a) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11: 296-310.

Van Der Heijden MGA, Bardgett RD, Van Straalen NM (2008b) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11: 296-310.

Van der Maarel E, Franklin J (2012) Vegetation ecology. John Wiley & Sons.

Van der Putten W, Kowalchuk G, Brinkman E, Doodeman G, Van der Kaaij R, Kamp A, Menting F, Veenendaal E (2007) Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. Ecology 88: 978-988.

Van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA (2013) Plant–soil feedbacks: the past, the present and future challenges. J Ecol 101: 265-276.

Van Grunsven RH, van der Putten WH, Bezemer T, Tamis WL, Berendse F, Veenendaal EM (2007) Reduced plant-soil feedback of plant species expanding their range as compared to natives. J Ecol 95: 1050-1057.

Vannette RL, Rasmann S (2012) Arbuscular mycorrhizal fungi mediate below-ground plant-herbivore interactions: a phylogenetic study. Funct Ecol 26: 1033-1042.

Wallis I, Watson M, Foley W (2002) Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. Aust J Zool 50: 507-519.

Wallis IR, Foley WJ (2005) The rapid determination of sideroxylonals in *Eucalyptus* foliage by extraction with sonication followed by HPLC. Phytochem Anal 16: 49-54.

Wallis IR, Nicolle D, Foley WJ (2010) Available and not total nitrogen in leaves explains key chemical differences between the eucalypt subgenera. For Ecol Manage 260: 814-821.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73: 5261-5267.

Wardle D, Bonner K, Barker G (2002) Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. Funct Ecol 16: 585-595.

Watanabe K, Murakami M, Hirao T, Kamata N (2014) Species diversity estimation of ambrosia and bark beetles in temperate mixed forests in Japan based on host phylogeny and specificity. Ecol Res 29: 299-307.

Wilcken CF, Raetano CG, Forti LC (2002) Termite pests in *Eucalyptus* forests of Brazil. Sociobiology 40: 179-190.

Williams J, Woinarski J (1997) Eucalypt ecology: individuals to ecosystems. Cambridge University Press, Cambridge, UK.

Williams K, Potts B (1996) The natural distribution of *Eucalyptus* species in Tasmania. Tasforests 8: 39-165.

Wingfield M, Slippers B, Hurley B, Coutinho T, Wingfield B, Roux J (2008) Eucalypt pests and diseases: growing threats to plantation productivity. South For 70: 139-144.

Wurst S, Wagenaar R, Biere A, Van der Putten WH (2010) Microorganisms and nematodes increase levels of secondary metabolites in roots and root exudates of *Plantago lanceolata*. Plant Soil 329: 117-126.

Xiang X, Gibbons SM, Yang J, Kong J, Sun R, Chu H (2015) Arbuscular mycorrhizal fungal communities show low resistance and high resilience to wildfire disturbance. Plant Soil 397: 347-356.

Xiang X, Shi Y, Yang J, Kong J, Lin X, Zhang H, Zeng J, Chu H (2014) Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. Sci Rep 4: 3829.

Yang J, Ci X, Lu M, Zhang G, Cao M, Li J, Lin L (2014) Functional traits of tree species with phylogenetic signal co-vary with environmental niches in two large forest dynamics plots. J Plant Ecol 7: 115-125.

Zhu Y, Comita LS, Hubbell SP, Ma K (2015) Conspecific and phylogenetic density-dependent survival differs across life stages in a tropical forest. J Ecol 103: 957–966. doi: 10.1111/1365-2745.12414.

Appendix A
Supplementary material to chapter 3

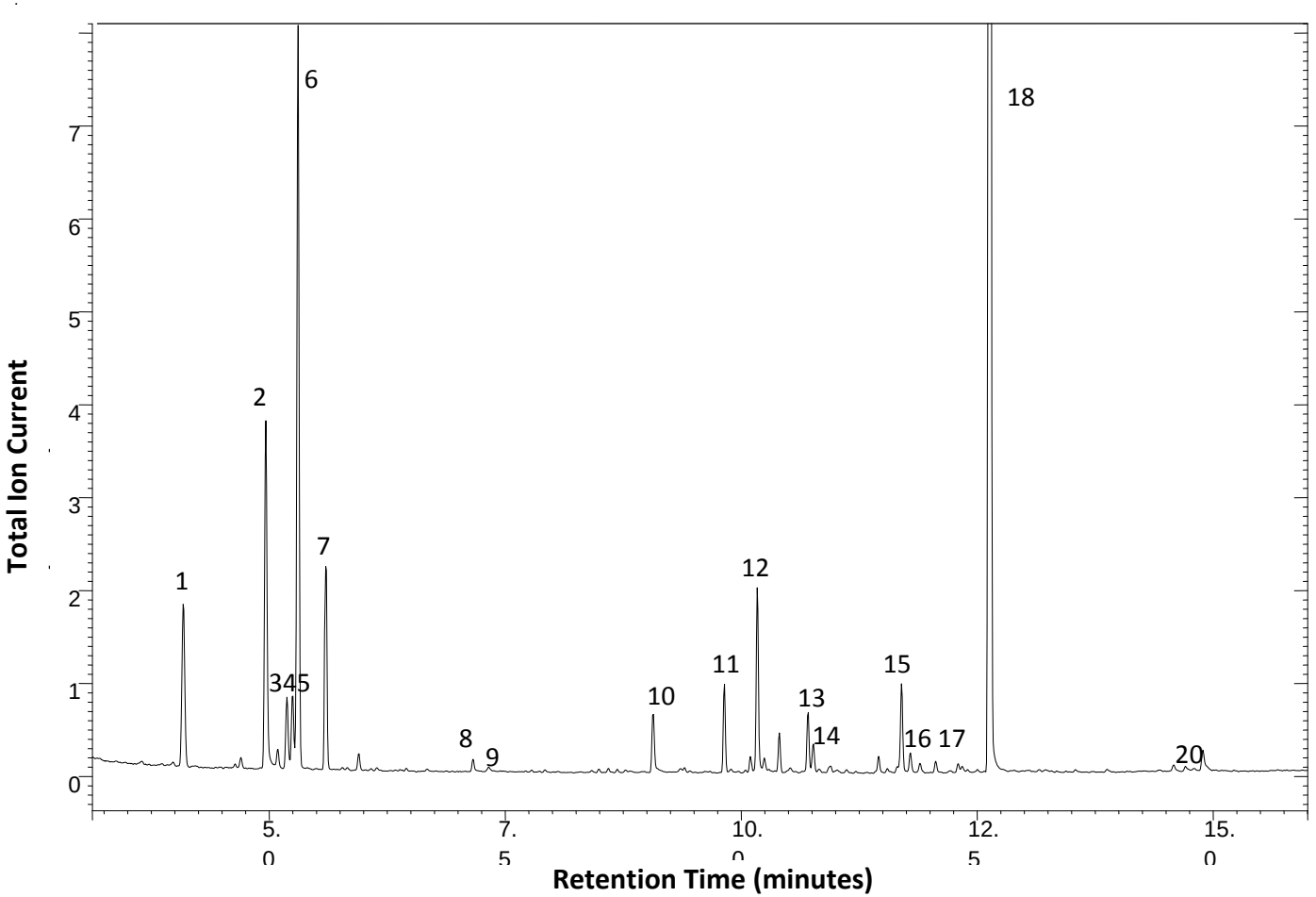
Table A1. Species display variable responses to heterospecific versus conspecific soils. The results of one-sample t-tests testing whether the mean response ratios (RR) of each species and trait significantly differed from zero as well as the slopes (β) of the linear relationships between the response ratios of each eucalypt species and phylogenetic distance between focal and conditioning species. Positive response ratios indicate greater species performance when inoculated with heterospecific conditioned soils compared with their own, while negative values indicate greater performance when inoculated with conspecific compared to heterospecific conditioned soils. Bold values represent significance, where * p = < 0.05, ** p = < 0.01 and *** p = <0.001.

		Variable											
		Survival		**Growth rate**		**Aboveground biomass**		**Belowground biomass**		**Total biomass**		**Root to shoot ratio**	
Subgenus	**Species**	**RR**	**β**	**RR**	**β**	**RR**	**β**	**RR**	**β**	**RR**	**β**	**RR**	**β**
Eucalyptus	***E. obliqua***	**0.07***	**0.06***	**0.08****	**0.06****	**0.24*****	**0.12****	**0.21*****	**0.12***	**0.23*****	**0.12****	**-0.08***	**0.07***
	E. pauciflora	**0.21*****	0.05	**0.07****	**0.06*****	**0.11****	**0.10****	**0.14*****	**0.09****	**0.12****	**0.10****	**0.20*****	0.01
	E. nitida	**0.08****	**0.07****	**0.08****	**0.06****	**0.20*****	**0.13*****	**0.18****	**0.14*****	**0.19*****	**0.13*****	0.02	**0.05***
	E. radiata	0.04	0.05	<0.01	**0.06***	**0.14****	**0.08***	**0.14****	**0.09***	**0.14****	**0.08***	**-0.08*****	0.01
	E. amygdalina	**0.35*****	**0.10****	**0.23*****	**0.06***	**0.25*****	0.06	**0.25*****	0.07	**0.25*****	0.06	0.04	0.01
Symphyomyrtus	***E. rodwayi***	**-0.14****	<-0.01	-0.04	**-0.05***	**-0.11****	-0.06	**-0.10****	-0.07	**-0.11****	-0.06	0.01	-0.02
	E. ovata	**-0.13****	-0.01	**0.06****	-0.04	**0.13****	-0.05	**0.14****	-0.05	**0.14****	-0.05	**0.14****	0.04
	E. barberi	-0.08	<0.01	0.01	-0.06	<0.01	-0.08	<0.01	-0.09	<0.01	-0.09	0.02	-0.02
	E. urnigera	**-0.18*****	-0.03	**-0.08*****	-0.03	**-0.21*****	-0.07	**-0.25*****	-0.07	**-0.22*****	-0.07	**-0.24*****	-0.02
	E. cordata	<-0.01	-0.01	0.05	-0.03	-0.06	-0.06	-0.08	-0.07	-0.06	-0.07	0.02	-0.03
	E. subcrenulata	-0.01	0.04	**-0.13*****	0.01	**0.07***	0.02	0.04	0.03	**0.06***	0.02	**-0.19*****	**0.08***
	E. globulus	0.02	0.04	0.03	0.02	<0.01	0.02	0.01	0.01	<0.01	0.02	0.05	-0.03
	E. dalrympleana	**0.06***	0.04	**-0.16*****	-0.03	**-0.22*****	-0.05	**-0.25*****	-0.07	**-0.23*****	-0.05	**-0.18*****	-0.05

Note: One-sample t-tests were conducted on the response ratios of each species and trait with 12 degrees of freedom and μ set at zero (i.e., no differential response to heterospecific versus conspecific soils) in R using the function t.test from the package *stats*.

Appendix B
Supplementary material to chapter 5

Figure B1. A typical gas chromatogram showing the terpene and acylphloroglucinol compounds detected in eucalypt roots.



	Compound	*Retention Time*
1	*α-pinene*	*4.092*
2	*α-phellandrene*	*4.964*
3	*α-terpinene*	*5.091*
4	*p-cymene*	*5.187*
5	*limonene*	*5.248*
6	*1,8-cineole*	*5.306*
7	*α-terpinene*	*5.600*
8	*terpinen-4-ol*	*7.159*
9	*α-terpineol*	*7.326*
10	*terpinyl acetate*	*9.070*
11	*α-gurjunene*	*9.822*
12	*aromadendrene*	*10.172*
13	*viridiflorene*	*10.709*
14	*bicyclogermacrene*	*10.764*
15	*globulol*	*11.700*
16	*viridiflorol*	*11.792*
17	*β-eudesmol*	*12.062*
18	*n-heptadecane (internal standard)*	*12.634*
19	*unknown acylphloroglucinol 1*	*14.582*
20	*unknown acylphloroglucinol 2*	*14.794*

Table B1. The arithmetic means and standard errors of the quantities of individual terpenes, acylphloroglucinols and FPCs detected in 24 eucalypt species. bolded values represent compounds detected above trace concentrations (≥0.1), concentrations between 0.01 and 0.1 were considered trace and compounds below trace concentrations were deemed absent (-).

			α-pinene		**α-phellandrene**		**α-terpinene**		**ρ-cymene**		**limonene**	
Subgenus	**Lineage**	**Species**	**Mean**	**±SE**	**Mean**	**±SE**	**Mean**	**±SE**	**Mean**	**±SE**	**Mean**	**±SE**
Eucalyptus	**1**	*E. obliqua*	-	na	-	na	-	na	-	na	-	na
	1	*E. regnans*	0.06	0.06	**0.12**	0.12	-	na	0.01	0.01	0.01	0.01
	1	*E. sieberi*	-	na	-	na	-	na	-	na	-	na
	1	*E. delegatensis*	-	na	-	na	-	na	-	na	-	na
	1	*E. pauciflora*	-	na	-	na	-	na	-	na	-	na
	2	*E. tenuiramis*	-	na	-	na	-	na	-	na	-	na
	2	*E. risdonii*	-	na	-	na	-	na	-	na	-	na
	2	*E. nitida*	-	na	-	na	-	na	-	na	-	na
	2	*E. radiata*	0.08	0.08	**0.10**	0.10	-	na	0.02	0.02	0.01	0.01
	2	*E. pulchella*	-	na	-	na	-	na	-	na	-	na
	2	*E. amygdalina*	-	na	-	na	-	na	-	na	-	na
Symphyomyrtus	**NA**	*E. gunnii*	-	na	-	na	-	na	-	na	-	na
	3	*E. brookeriana*	0.01	<0.01	0.01	0.01	-	na	-	na	-	na
	3	*E. rodwayi*	0.04	0.03	0.09	0.09	-	na	0.03	0.03	0.01	0.01
	3	*E. barberi*	0.04	0.04	**0.16**	0.10	0.01	0.01	0.02	0.02	0.01	0.01
	4	*E. urnigera*	0.01	<0.01	-	na	-	na	-	na	-	na
	4	*E. cordata*	**0.25**	0.03	**0.26**	0.01	0.02	<0.01	0.05	0.01	0.02	<0.01
	4	*E. subcrenulata*	0.08	0.01	**0.25**	0.02	0.02	<0.01	0.06	<0.01	0.02	<0.01
	4	*E. johnstonii*	-	na	-	na	-	na	-	na	-	na
	NA	*E. globulus*	0.06	0.02	0.09	0.01	0.01	<0.01	0.01	<0.01	0.01	<0.01
	5	*E. perriniana*	**0.10**	0.01	**0.12**	0.07	-	na	0.02	0.01	0.01	<0.01
	5	*E. dalrympleana*	-	na	-	na	-	na	-	na	-	na
	5	*E. viminalis*	0.04	0.04	**0.12**	0.03	0.01	0.01	0.02	0.01	0.02	0.01
	5	*E. rubida*	0.04	0.04	**0.12**	0.12	0.01	0.01	0.02	0.02	0.01	0.01

All compounds are expressed as mg g^{-1} DM 1,8-cineole equivalents.

Table B1. (cont.)

Subgenus	Lineage	Species	1,8-cineole Mean	1,8-cineole ±SE	γ-terpinene Mean	γ-terpinene ±SE	terpinene-4-ol Mean	terpinene-4-ol ±SE	α-terpineol Mean	α-terpineol ±SE	terpinyl acetate Mean	terpinyl acetate ±SE
Eucalyptus	**1**	*E. obliqua*	0.01	<0.01	-	na	-	na	-	na	-	na
	1	*E. regnans*	0.06	0.06	-	na	-	na	-	na	0.01	0.01
	1	*E. sieberi*	0.01	<0.01	-	na	-	na	-	na	-	na
	1	*E. delegatensis*	-	na	-	na	-	na	-	na	-	na
	1	*E. pauciflora*	-	na	-	na	-	na	-	na	-	na
	2	*E. tenuiramis*	0.01	<0.01	-	na	-	na	-	na	-	na
	2	*E. risdonii*	0.01	<0.01	-	na	-	na	-	na	-	na
	2	*E. nitida*	0.01	<0.01	-	na	-	na	-	na	-	na
	2	*E. radiata*	**0.11**	0.10	0.01	0.01	-	na	-	na	-	na
	2	*E. pulchella*	0.02	0.01	-	na	-	na	-	na	-	na
	2	*E. amygdalina*	0.01	na	-	na	-	na	-	na	-	na
Symphyomyrtus	**NA**	*E. gunnii*	0.01	0.01	-	na	-	na	-	na	-	na
	3	*E. brookeriana*	0.01	na	-	na	-	na	-	na	-	na
	3	*E. rodwayi*	**0.11**	0.09	0.04	0.04	-	na	-	na	0.05	0.05
	3	*E. barberi*	**0.15**	0.14	0.05	0.05	-	na	-	na	0.01	0.01
	4	*E. urnigera*	0.01	<0.01	-	na	-	na	-	na	-	na
	4	*E. cordata*	**0.28**	0.08	**0.11**	0.05	0.01	0.01	0.02	0.01	-	na
	4	*E. subcrenulata*	**0.18**	0.02	0.06	0.01	0.01	<0.01	0.01	<0.01	0.07	0.01
	4	*E. johnstonii*	0.01	<0.01	-	na	-	na	-	na	-	na
	NA	*E. globulus*	0.09	0.03	0.05	0.02	-	na	0.01	<0.01	-	na
	5	*E. perriniana*	**0.13**	0.01	-	na	-	na	-	na	0.01	<0.01
	5	*E. dalrympleana*	0.01	<0.01	-	na	-	na	-	na	-	na
	5	*E. viminalis*	**0.16**	0.15	0.05	0.05	-	na	-	na	0.01	0.01
	5	*E. rubida*	0.08	0.08	0.02	0.02	-	na	-	na	0.03	0.03

All compounds are expressed as mg g^{-1} DM 1,8-cineole equivalents.

Table B1. (cont.)

Subgenus	Lineage	Species	α-gurjunene		aromadendrene		viridiflorene		bicyclogermacrene		globulol	
			Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
Eucalyptus	**1**	*E. obliqua*	-	na	-	na	-	na	-	na	-	na
	1	*E. regnans*	0.01	0.01	0.06	0.05	0.01	0.01	-	na	0.02	0.02
	1	*E. sieberi*	-	na	-	na	-	na	-	na	-	na
	1	*E. delegatensis*	-	na	-	na	-	na	-	na	-	na
	1	*E. pauciflora*	-	na	-	na	-	na	-	na	-	na
	2	*E. tenuiramis*	-	na	-	na	-	na	-	na	-	na
	2	*E. risdonii*	-	na	-	na	-	na	-	na	-	na
	2	*E. nitida*	-	na	-	na	-	na	-	na	-	na
	2	*E. radiata*	0.01	0.01	0.02	0.02	0.01	0.01	-	na	0.01	0.01
	2	*E. pulchella*	-	na	-	na	-	na	-	na	-	na
	2	*E. amygdalina*	-	na	-	na	-	na	-	na	-	na
Symphyomyrtus	**NA**	*E. gunnii*	-	na	-	na	-	na	-	na	-	na
	3	*E. brookeriana*	-	na	-	na	-	na	-	na	-	na
	3	*E. rodwayi*	0.02	0.02	0.04	0.04	0.01	0.01	0.01	0.01	0.02	0.02
	3	*E. barberi*	0.02	0.02	0.05	0.05	0.01	0.01	0.01	0.01	0.02	0.02
	4	*E. urnigera*	-	na	-	na	-	na	-	na	-	na
	4	*E. cordata*	0.06	<0.01	**0.30**	0.01	0.07	<0.01	0.03	0.01	**0.13**	<0.01
	4	*E. subcrenulata*	0.03	<0.01	0.04	0.03	0.01	0.01	0.01	<0.01	0.04	<0.01
	4	*E. johnstonii*	-	na	-	na	-	na	-	na	-	na
	NA	*E. globulus*	0.02	<0.01	0.05	0.05	0.02	<0.01	0.02	0.01	0.04	0.01
	5	*E. perriniana*	0.02	0.01	0.06	0.04	0.01	0.01	0.01	<0.01	0.03	0.02
	5	*E. dalrympleana*	-	na	-	na	-	na	-	na	-	na
	5	*E. viminalis*	0.02	0.02	0.04	0.04	-	na	0.01	0.01	0.02	0.02
	5	*E. rubida*	0.01	0.01	0.03	0.03	0.01	0.01	-	na	0.01	0.01

All compounds are expressed as mg g^{-1} DM 1,8-cineole equivalents.

Table B1. (cont.)

			viridiflorol		β-eudesmol		unknown 1		unknown 2	
Subgenus	**Lineage**	**Species**	**Mean**	**±SE**	**Mean**	**±SE**	**Mean**	**±SE**	**Mean**	**±SE**
Eucalyptus	**1**	*E. obliqua*	-	na	-	na	-	na	-	na
	1	*E. regnans*	-	na	-	na	0.01	0.01	-	na
	1	*E. sieberi*	-	na	-	na	-	na	-	na
	1	*E. delegatensis*	-	na	-	na	-	na	-	na
	1	*E. pauciflora*	-	na	-	na	-	na	-	na
	2	*E. tenuiramis*	-	na	-	na	-	na	-	na
	2	*E. risdonii*	-	na	-	na	-	na	-	na
	2	*E. nitida*	-	na	-	na	-	na	-	na
	2	*E. radiata*	-	na	-	na	0.03	0.03	-	na
	2	*E. pulchella*	-	na	-	na	-	na	-	na
	2	*E. amygdalina*	-	na	-	na	-	na	-	na
Symphyomyrtus	**NA**	*E. gunnii*	-	na	-	na	-	na	-	na
	3	*E. brookeriana*	-	na	-	na	-	na	-	na
	3	*E. rodwayi*	-	na	-	na	0.01	0.01	0.01	0.01
	3	*E. barberi*	0.01	0.01	-	na	-	na	-	na
	4	*E. urnigera*	-	na	-	na	-	na	-	na
	4	*E. cordata*	0.03	<0.01	0.02	<0.01	0.01	<0.01	0.01	<0.01
	4	*E. subcrenulata*	0.01	<0.01	0.01	<0.01	0.01	<0.01	0.01	<0.01
	4	*E. johnstonii*	-	na	-	na	-	na	-	na
	NA	*E. globulus*	0.01	<0.01	0.01	<0.01	-	na	-	na
	5	*E. perriniana*	0.01	<0.01	-	na	0.03	<0.01	-	na
	5	*E. dalrympleana*	-	na	-	na	-	na	-	na
	5	*E. viminalis*	-	na	-	na	-	na	-	na
	5	*E. rubida*	-	na	-	na	-	na	-	na

All compounds are expressed as mg g^{-1} DM 1,8-cineole equivalents.

Table B1. (cont.)

Subgenus	Lineage	Species	Sideroxylonal A Mean	±SE	Sideroxylonal C Mean	±SE	Macrocarpal A Mean	±SE	unknown FPC Mean	±SE	other FPCs Mean	±SE
Eucalyptus	**1**	*E. obliqua*	-	na	-	na	-	na	-	na	-	na
	1	*E. regnans*	-	na	-	na	-	na	0.01	0.01	-	na
	1	*E. sieberi*	-	na	-	na	-	na	-	na	-	na
	1	*E. delegatensis*	-	na	-	na	-	na	-	na	-	na
	1	*E. pauciflora*	-	na	-	na	-	na	0.01	0.01	-	na
	2	*E. tenuiramis*	-	na	-	na	-	na	0.01	0.01	-	na
	2	*E. risdonii*	-	na	-	na	-	na	-	na	-	na
	2	*E. nitida*	0.01	0.01	0.01	<0.01	-	na	-	na	-	na
	2	*E. radiata*	-	na	-	na	-	na	-	na	-	na
	2	*E. pulchella*	-	na	-	na	-	na	-	na	-	na
	2	*E. amygdalina*	-	na	-	na	-	na	0.02	0.02	-	na
Symphyomyrtus	**NA**	*E. gunnii*	-	na	-	na	-	na	-	na	-	na
	3	*E. brookeriana*	0.05	0.02	0.05	0.02	0.02	0.01	**0.36**	0.09	**0.59**	0.28
	3	*E. rodwayi*	0.05	0.02	0.05	0.02	0.02	0.01	0.05	0.02	-	na
	3	*E. barberi*	**0.11**	<0.01	**0.10**	0.01	0.05	<0.01	**2.33**	0.15	**2.64**	0.47
	4	*E. urnigera*	-	na	-	na	-	na	-	na	-	na
	4	*E. cordata*	-	na	-	na	-	na	-	na	-	na
	4	*E. subcrenulata*	0.01	0.01	0.01	0.01	-	na	0.01	0.01	-	na
	4	*E. johnstonii*	0.01	0.01	-	na	-	na	-	na	-	na
	NA	*E. globulus*	-	na	-	na	-	na	-	na	-	na
	5	*E. perriniana*	**0.18**	0.03	0.09	0.01	**0.11**	0.02	**1.50**	0.10	**3.74**	0.59
	5	*E. dalrympleana*	**0.35**	0.09	**0.17**	0.06	**0.24**	0.07	**1.35**	0.05	**5.96**	0.53
	5	*E. viminalis*	**0.46**	0.01	**0.23**	0.07	**0.31**	0.04	**2.08**	0.14	**7.61**	0.82
	5	*E. rubida*	**0.92**	0.08	**0.86**	0.05	**0.44**	0.08	**5.15**	0.38	**8.64**	1.46

All FPCs are expressed as mg g^{-1} DM.

Appendix C
Supplementary material to chapter 6

Table C1. Results of two-tailed Kendall rank correlations analysing for relationships between root chemicals and the proportion of pots of each species in which each fungal family was detected. Bold values indicate statistical significance at $\alpha = 0.05$.

	Total phenolics		**Condensed tannins**		**Total terpenes**		**Total FPCs**	
Fungal families	tau	P	tau	P	tau	P	tau	P
Fistulina	0.31	0.243	-0.06	0.867	-0.62	**0.018**	-0.15	0.614
Unclassified Microascales	0.45	0.082	-0.04	0.934	-0.45	0.094	-0.15	0.614
Unclassified Hysterangiales	-0.17	0.532	0.21	0.434	0.62	**0.018**	0.16	0.581
Davidiellaceae	-0.31	0.238	0.16	0.555	0.09	0.766	0.18	0.503

Note: Correlations examined species-level data from chapters 4 and 5 in R using the Kendall function from the Package Kendall.